Canada

Fisheries and Oceans | Pêches et Océans

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Government of Canada
Fisheries and Oceans

Scientific Information
and Publications Branch
Ottawa K1A 0E6

Gouvernement du Canada
Pêches et Océans

Direction de l'information
et des publications scientifiques
Ottawa K1A 0E6

The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79.

Editorial policy

The *Journal* publishes original research articles and notes, critical reviews, Perspectives (essays of opinion or hypothesis), comments, and book reviews. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or synthesis, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmatory state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

It would assist the Editors if prospective authors identified briefly by covering letter (a) aspects of their papers that meet the foregoing objectives, (b) potential referees, and (c) other manuscripts contemplated or in press containing the same or similar information.

Submissions in English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company.

A charge is made for manuscripts published. When submitting a manuscript, an author must state that the charge will be accepted. See recent issues of the Journal for current page charges.

Guides

Guides for authors and typists appear in the first issue of each volume and are available free from the *Journal*.

Subscriptions

The *Journal* is issued monthly to form annual volumes of twelve numbers and Supplements. Orders and inquiries regarding subscriptions must be sent directly to Supply and Services Canada, Ottawa, Ontario, Canada, K1A 0S9. Prepayment is required in Canadian funds to the order of "The Receiver General for Canada."

Microfilm

Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–79.

Politique de rédaction

Le *Journal* publie des articles et des notes fondés sur une recherche originale, des critiques, des essais portant sur une opinion ou une hypothèse (Perspectives), des commentaires et des comptes rendus de livres. Les textes peuvent avoir trait aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatique. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. Ils doivent clairement démontrer qu'ils contribuent aux connaissances en faisant plus que corroborer des faits. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles.

Les auteurs éventuels aideraient les rédacteurs s'ils identifiaient brièvement, dans une lettre d'accompagnement (a) les aspects de leurs textes qui répondent particulièrement aux objectifs indiqués ci-dessus, (b) des arbitres possibles et (c) d'autres manuscrits envisagés ou sous presse, dont la teneur est identique ou se rapproche de celui qui est soumis.

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le *Journal* décline toute responsabilité quant aux énoncés des contributeurs. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

Les manuscrits acceptés sont sujets à des frais de publication. Quant un auteur présente un texte, il doit préciser que ces frais seront acceptés. On trouvera dans une récente livraison du *Journal* les taux par page en vigueur.

Guides

Le guide des auteurs et celui des secrétaires d'auteurs paraissent dans la première livraison de chaque volume et sont disponibles gratuitement du *Journal*.

Abonnement

Le *Journal* paraît tous les mois, formant ainsi un volume de douze numéros et Suppléments par année. On est prié d'adresser directement commandes et demande de renseignements relatifs à l'abonnement à Approvisionnements et Services Canada, Ottawa (Ontario), Canada K1A 0S9. Les paiements doivent être faits à l'avance en devises canadiennes, à l'ordre du « Receveur général du Canada ».

Microfilms

On peut acheter sur microfilms de 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, É.-U.

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 42, No. 12, December 1985 | **Volume 42, nº 12, décembre 1985**

Scientific Information and Publications Branch/Direction de l'information et des publications scientifiques

J. Watson, Ph.D.	*Director and Editor-in-Chief/Directeur et rédacteur en chef*
Johanna M. Reinhart, M.Sc.	*Deputy Director and Editor/Sous-directrice et rédactrice*
D. G. Cook, Ph.D./Lorraine C. Smith, Ph.D.	*Assistant Editors/Rédacteurs adjoints*
J. Camp/G. J. Neville/P. M. Burke/M. L. Gilbert	*Editorial and Publishing Services/Services de rédaction et d'édition*
R. O. Brinkhurst, Ph.D./J. R. M. Kelso, Ph.D.	*Book Review Editors/Rédacteurs des comptes rendus*

Editorial Board/Conseil de rédaction

L. M. Dickie	*Department of Fisheries and Oceans, Dartmouth*
P. J. Dillon	*Ontario Ministry of the Environment*
J. J. Dodson	*University of Laval*
M. C. Healey	*Department of Fisheries and Oceans, Nanaimo*
L. Legendre	*Université Laval*
J. J. Magnuson	*University of Wisconsin*
M. Sinclair	*Department of Fisheries and Oceans, Halifax*
S. J. Smith	*Department of Fisheries and Oceans, Dartmouth*
J. Sprague	*University of Guelph*
C. J. Walters	*University of British Columbia*
C. M. Wood	*McMaster University*
A. P. Zimmerman	*University of Toronto*

Editorial Office:
Scientific Information
and Publications Branch
Department of Fisheries and Oceans
Centennial Towers
200 Kent Street
Ottawa, Canada K1A 0E6

Bureau de rédaction :
Direction de l'information
et des publications scientifiques
Ministère des Pêches et des Océans
Les Tours Centennial
200, rue Kent
Ottawa, Canada K1A 0E6

The *Journal* is abstracted or indexed in: / Le *Journal* est résumé ou signalé dans :

Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, and Science Citation Index

Estimating the Standing Biomass of Aquatic Macrophytes[1]

John A. Downing

Département de Sciences Biologiques, Université de Montréal, CP 6128, Succursale "A", Montréal, Qué. H3C 3J7

and M. Robin Anderson[2]

Department of Biology, McGill University, 1205 Avenue Dr. Penfield, Montreal, Que. H3A 1B1

Downing, J. A., and M. R. Anderson. 1985. Estimating the standing biomass of aquatic macrophytes. Can. J. Fish. Aquat. Sci. 42: 1860–1869.

The accuracy, precision, and cost-efficiency of estimation techniques for macrophyte standing biomass are examined, and solutions are offered to problems encountered in the design of efficient sampling programs. Five sizes of quadrats (100 cm^2 to 1 m^2) were compared on seven occasions and all five yielded equivalent biomass estimates in six of these tests. Published and unpublished values of biomass sampling variance measured around the world were predictable from average standing biomass and size of sampler. The sampling cost was predictable from sampler size. Analysis of these relationships allows optimization of sampling design. The use of small quadrat samplers with great replication is recommended. Adherence to this protocol can result in a 30-fold reduction in sampling cost. Recommendations are compared with techniques actually used by aquatic ecologists. A brief analysis of spatial heterogeneity of aquatic macrophyte biomass is presented.

On examine l'exactitude, la précision et le rapport coût-efficacité de techniques d'estimation de la biomasse des macrophytes et l'on propose des solutions aux problèmes rencontrés lors de la conception de programmes d'échantillonnage efficaces. On a comparé cinq grandeurs de quadrats (de 100 cm^2 à 1 m^2) à sept reprises, et les cinq ont donné des estimations de biomasse équivalentes dans six cas. Les valeurs publiées et inédites de la variance de l'échantillonnage de la biomasse calculées dans le monde entier étaient prévisibles à partir de la biomasse moyenne et de la dimension de l'échantillonneur. Le coût d'échantillonnage était prévisible à partir de la dimension de l'échantillonneur. L'analyse de ces relations permet d'optimiser la conception de programmes d'échantillonnage. On recommande d'utiliser des échantillonneurs pour petits quadrats en répétant l'opération de nombreuses fois. En suivant ce protocole, on peut réduire de 30 fois le coût d'échantillonnage. On compare les recommandations formulées avec les techniques effectivement employées par les écologistes aquatiques. On présente aussi une analyse succincte de l'hétérogénéité spatiale de la biomasse des macrophytes aquatiques.

Received April 9, 1984
Accepted July 30, 1985
(J7752)

Reçu le 9 avril 1984
Accepté le 30 juillet 1985

Recent research underscores the importance of aquatic macrophytes to the functioning of freshwater ecosystems (e.g. Carignan and Kalff 1982; Cattaneo 1983; Howard-Williams and Allanson 1981; Jacoby et al. 1982; Landers 1982). Assessments of biomass, production, nutrient cycling, and community dynamics all rely on estimates of macrophyte standing stock (Landers 1982; Westlake 1965, 1969). In addition, the estimation of macrophyte standing biomass is essential to studies of the epiphytic invertebrate fauna (Downing 1984; Downing and Cyr 1985), and these measurements are in turn necessary for the assessment of fish food resources in lakes (e.g. George and Hadley 1979; Laughlin and Werner 1980). Precise measurement of these factors is limited by the heterogeneity of macrophyte beds and resultant sampling errors. In spite of the importance of macrophyte biomass estimation, no systematic evaluation of estimation techniques has been made.

Standing macrophyte biomass is usually estimated by quantitative harvest of macrophytes contained in randomly placed samplers of known area. Direct harvest of macrophytes from quadrats provides the most accurate estimates of standing biomass (Grace and Tilley 1976; Rich et al. 1971; Sheldon and Boylen 1978; Westlake 1969), although grabs (e.g. Unni 1977) and hooks (e.g. Bayley et al. 1978) have sometimes been used. Macrophytes are usually collected by SCUBA divers because they can see whether plants have been missed, can avoid entangling plants not growing within the quadrat area, and loss rates are very small.

The field researcher must decide what size of quadrat to employ, and how many replicate samples to take for each biomass estimate. The size of quadrat is important because it may affect both the accuracy and precision of macrophyte biomass estimates. Terrestrial ecologists have concluded that small quadrats yield overestimates of plant biomass due to edge effects (Hanson 1930; Hendricks 1956; Sukhatme 1947; Van Dyne et al. 1963; Wiegert 1962), and large quadrats yield higher precision for the same level of replication (Pechanec and Stewart 1940; Smith 1938). While many ecologists have used large samplers (Green 1979, p. 39), other researchers have

[1]A joint publication of the Groupe d'Ecologie des Eaux douces of l'Université de Montréal and the McGill University Limnology Research Centre.

[2]Present address: Département des Sciences Biologiques, Université du Québec à Montréal, CP 8888, Succursale "A", Montréal, Qué. H3C 3P8.

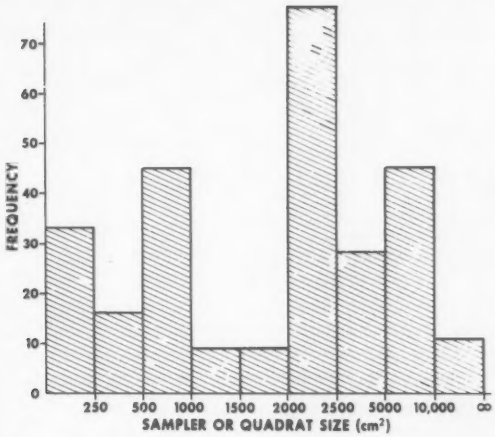


FIG. 1. Sampler sizes used for aquatic macrophytes in studies published between 1970 and 1984 in 30 well-known aquatic journals. If studies used a range of sampler sizes, both the upper and lower limits of sampler size range are included in the histogram. The bars include observations made at their upper extreme but not their lower extreme. Note unequal quadrat size intervals.

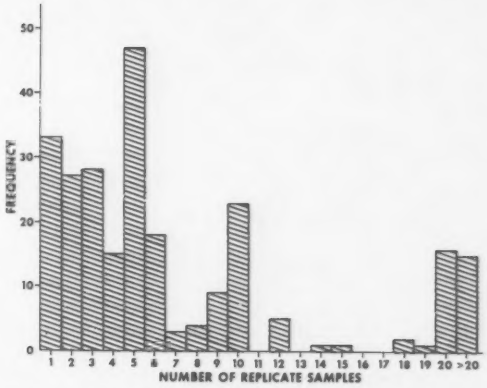


FIG. 2. Number of replicate samples (n) taken to estimate aquatic macrophyte biomass in studies published between 1970 and 1984 in 30 well-known aquatic journals. If studies used a range of n, both the upper and lower limits of the range are included in the histogram.

suggested that small samples are the most cost-effective (Green 1979, p. 38–39; John et al. 1980; Pechanec and Stewart 1940; Smith 1938; Ursic and McClurkin 1959). Aquatic ecologists have sampled macrophytes using a wide variety of sampler sizes (Fig. 1; median 2500 cm^2). Aquatic methods manuals and handbooks offer little help in choice of quadrat size (e.g. Westlake 1969, p. 26): suggested quadrat areas are 333 cm^2 (Schwoerbel 1970, p. 80), 1332 cm^2 (Welch 1948, p. 330), 2500–5000 cm^2 (Lind 1979, p. 178; Wetzel and Likens 1979, p. 266), or 400 cm^2 to 1 m^2 (Wood 1975, p. 19). The effect of quadrat size on the accuracy and precision of aquatic macrophyte biomass estimates is unknown.

The number of replicate samples (n) to be taken depends upon the spatial distribution and the desired level of precision of the variable to be estimated. Green (1979, p. 39) suggests that "The best sample number is the largest number..." because the standard error (SE) of the mean is $\sqrt{s^2/n}$, where s^2 is the sampling variance. In practice, sampling cost limits sample number (Cochran 1977, p. 84), and it is therefore important to know in advance the level of replication that will yield an acceptable level of precision. Methods manuals offer no rules-of-thumb, because n can be calculated for a desired level of precision only if sampling variance is known or can be predicted (Cochran 1977, p. 243; Elliott 1977, p. 129). A priori, we might guess that spatial variation of macrophyte biomass follows a Poisson distribution or the negative binomial family like those found for benthic invertebrate populations (Elliott 1977, chap. 5). Although macrophyte biomass is thought to be spatially aggregated (Davies 1970; Westlake 1969; Wong and Clark 1979), no general model has been advanced to predict the expected variance for replicate macrophyte biomass estimates. Not surprisingly, aquatic ecologists have employed a wide variety of sample replication (Fig. 2).

Concrete decision rules for macrophyte biomass sampling programs are needed in order to increase statistical power and eliminate wasted effort. The purpose of this study is to compare the accuracy, precision, and relative cost of harvest techniques for the measurement of aquatic macrophyte biomass. We present the results of side-by-side comparisons of the accuracy of quadrats of different sizes, and provide means of predicting sampling precision and optimizing macrophyte biomass sampling programs.

Methods

Accuracy

Comparisons of the relative accuracy of various quadrat sizes for the estimation of total aboveground standing macrophyte biomass were made on seven occasions during 1982 and 1983. Comparisons in 1982 were made during July and August in Lake Memphremagog, Quebec–Vermont (45°0′N, 72°17′W), near Cove Island. The substrate was sandy clay to clayey alluvium, and dominant macrophyte species were *Cabomba caroliniana* (Gray), *Elodea canadensis* (Michx.), *Heteranthera dubia* ((Jacq.) MacM.), *Myriophyllum spicatum* (L.), *Najas* sp., *Potamogeton richardsonii* ((Benn.) Rydb.), and *Vallisneria americana* (Michx.). Four further comparisons were made in the same geographical area during 1983 using greater replication and a wider range of total biomass levels and substrate types. From lowest to highest macrophyte biomass, these sites were as follows: (1) Lake Orford, on sandy substrate supporting *Chara* sp., *E. canadensis*, *Isoetes* sp., *Najas* sp., *Nitella* sp., *Potamogeton crispus* (L.), and *P. praelongus* (Wulf.); (2) South Lake Memphremagog, on sandy substrate supporting *E. canadensis*, *Eriocaulon septangularis* (With.), *H. dubia*, *Isoetes* sp., *M. spicatum*, *Najas* sp., *Potamogeton gramineus* (L.), and *V. americana*; (3) North Lake Memphremagog, on mud and clay supporting *C. caroliniana*, *E. septangularis*, *H. dubia*, *M. spicatum*, *Najas* sp., *Nitella* sp., *P. gramineus*, *P. praelongus*, *P. robbinsii* (Oakes), and *V. americana*; and (4) Lake Massawippi, on mud supporting a monospecific stand of *M. spicatum*. Comparisons in 1983 were all made near maximum seasonal biomass, at the end of August.

The five quadrat sizes compared covered a geometric series from 100 cm^2 to 1 m^2. This range includes over 90% of the sampler sizes used in the published literature (Fig. 1). Samples were collected using square quadrats made of (1982) steel or (1983) PVC pipe (J. A. Downing, unpubl.). At each site, the quadrats were placed at randomly drawn points along either side of a numbered 50-m line stretched at uniform depth across the

TABLE 1. Sources of data on spatial variation in macrophyte biomass used to derive the variance function (n' indicates the number of sets of replicate observations gathered from each source).

Source	Water body	Location	n'
M. R. Anderson, unpubl.	26 sites in 8 lakes[a]	Quebec, Canada	206
J. A. Downing and M. R. Anderson, unpubl.	4 sites in 3 lakes[a]	Quebec, Canada	112
J. A. Downing and H. Cyr, unpubl.	Lake Memphremagog[b]	Quebec, Canada	55
Engel 1982	Marion Millpond	Wisconsin, USA	12
Howard-Williams 1978	Swartvlei Lake	South Africa	11
Howard-Williams 1980	Wilderness Lakes	South Africa	6
C. Howard-Williams, pers. comm.[c]	Langvlei Lake	South Africa	2
C. Howard-Williams, unpubl.	Lake Sibaya	Africa	6
Howard-Williams and Allanson 1978	Swartvlei Lake	South Africa	27
Howard-Williams and Allanson 1981	Swartvlei Lake	South Africa	1
C. Howard-Williams and J. Davis, unpubl.	Lake Waikaremoana[d]	New Zealand	77
Howard-Williams and Lenton 1975	Lake Chilwa	Malawi, Africa	2
Howard-Williams and Liptrot 1980	Swartvlei Estuary	South Africa	4
Howard-Williams et al. 1982	Whangamata Stream	New Zealand	50
Jacoby et al. 1982	Long Lake	Washington, USA	12
T. Kiørboe, unpubl.[e]	Ringkøbing Fjord[f]	Denmark	560
Kirkman et al. 1982	Port Hocking	NSW, Australia	8
Landers 1982; pers. comm.[g]	Lake Monroe	Indiana, USA	4
Menzie 1979	Hudson River	New York, USA	8
Nicholson and Aroyo 1973	Chatauqua Lake	New York, USA	1
Sahai and Sinha 1970	Lake Ramgarh	Gorakhpur, India	12
Welch et al. 1982	Long Lake	Washington, USA	24

[a]Covering a wide range of ecotypes.
[b]Several different dates.
[c]Department of Scientific and Industrial Research, Taupo, New Zealand.
[d]21 sampling sites.
[e]The Nature Scientific Reserve, Tipperne, Denmark.
[f]99 sampling sites.
[g]S.U.N.Y., Oswego, NY.

macrophyte bed. We took between 3 and 26 replicate samples with each quadrat size at each site. The number of quadrats used in 1983 was adjusted to yield a precision of $\text{SE}/\bar{x} = 0.1$ (SE = standard error; $\bar{x}$ = mean biomass in grams dry weight per square metre), determined by a preliminary sampling survey. Macrophytes were harvested from within the quadrats by divers using SCUBA. Plants were cut with grass-clippers or broken directly at the sediment surface and then were placed carefully in 100-μm-mesh nylon bags. Six different divers were assigned samples randomly. Divers collected all plants rooted within the quadrats and every second plant found exactly on the edge of the quadrats. In practice, plants were usually clearly in or out of the quadrat.

After collection, samples were washed, sorted by species (for other analyses), air dried, dried to constant weight at 80°C (48 h), and weighed (±0.1 mg). Weights of all species found growing within a quadrat were summed to yield total aboveground dry weight for each replicate quadrat. These dry weights were transformed to estimated dry weight per square metre, and statistical comparisons among all sizes of quadrats for each of the seven experiments were made using the nonparametric Kruskal–Wallis one-way analysis of variance (Conover 1971, p. 256).

Precision

The precision of samplers of different sizes has been analyzed by finding the general empirical relationship between sampling variance (s^2), size of mean observation ($\bar{x}$, i.e. biomass), and size of sampling unit (A square centimetres) (Downing 1979; Downing and Cyr 1985). This relation can be rearranged to predict the number of quadrats of a given size that one must take at a given biomass level to obtain an acceptable level of precision.

We have built such a variance function from our data and those supplied to us by macrophyte ecologists throughout the world (Table 1). The data collected consisted of arithmetic means ($\bar{x}$), $n - 1$ weighted variances (s^2), and quadrat sizes (A) for aquatic macrophytes from lakes, streams, rivers, estuaries, and oceans. Data on total macrophyte biomass and aboveground macrophyte biomass were included for comparative purposes. Data on $\bar{x}$ (grams dry weight per square metre) and s^2 (calculated on a per square metre basis) of both single species and total macrophyte community biomass were also analyzed. The model

$$(1) \quad \log s^2 = \log a + b \log \bar{x} + c \log A,$$

where ($\log a$), b, and c are fitted constants, was fitted to the full dataset using regression analysis (Draper and Smith 1981; Nie et al. 1975). The residuals were plotted against the independent variables to make certain that the regression model fitted the data well, and that no systematic lack of fit remained after analysis. Comparisons of the fit of subsets of the data (e.g. species or categories) were made using parametric and nonparametric methods.

Cost

The optimal combination of sampler size (A) and sample number (n) was determined by measuring the cost (in time) of sampling under various sampling conditions. The primary cost

TABLE 2. Average biomass (g dry wt.·m^{-2}) estimated using five different quadrat sizes on seven different occasions. Means were calculated after fourth-root transformation. χ^2 values are for the Kruskal–Wallis test (Conover 1971, p. 256). Number of samples is indicated in parentheses. P is the significance level for rejection of the hypothesis that all quadrats in the row yield identical distributions of untransformed estimated macrophyte biomass.

	Quadrat size (cm^2)						
Site	100	316	1000	3162	10 000	χ^2	P
Lake Orford (1983)	5.3 (26)	4.4 (24)	2.9 (19)	5.9 (20)	6.2 (9)	4.5	0.34
Cove Island (1982), Exp. 1[a]	23.2 (6)	15.0 (4)	16.0 (6)	10.2 (4)	9.9 (3)	2.7	0.60
Cove Island (1982), Exp. 5[a]	50.5 (10)	—	55.7 (8)	—	53.3 (5)	0.1	0.95
Cove Island (1982), Exp. 8[a]	54.5 (20)	—	51.7 (20)	—	43.2 (12)	1.6	0.45
Memphremagog South (1983)	65.1 (21)	67.8 (18)	71.7 (15)	74.7 (13)	63.2 (8)	2.3	0.68
Memphremagog North (1983)	171.7 (13)	140.0 (11)	183.4 (10)	129.0 (9)	138.4 (5)	4.3	0.37
Lake Massawippi (1983)	0.9 (11)	50.8 (10)	189.5 (8)	166.1 (7)	243.4 (4)	15.2	<0.01

[a]Quadrat sizes were 112, 569, 991, 2540, and 10 002 cm^2.

in estimating total standing macrophyte biomass is the time that it takes to collect the macrophytes. In our comparisons of quadrat accuracy, each diver used a waterproof chronograph to record the time (T, minutes) elapsed between the start and finish of macrophyte collection from each quadrat. A nonlinear function was fitted to these data using the Marquardt (1963) method (Robinson 1977):

$$(2) \quad T = q(1 - e^{-dA})$$

where e is the base of natural logarithms, A is the quadrat size (square centimetres), and d and q are fitted constants. The residuals were examined for lack of fit. Predicted costs were then combined with predicted numbers of samples to determine the most cost-efficient method of attaining a given precision.

Taxonomic nomenclature for our collections follows Fassett (1966), while species names for other data are those supplied by their authors (Table 1).

Results and Discussion

Accuracy

We took over 350 samples to determine the relative accuracy of different quadrat sizes for the estimation of aboveground standing biomass. These comparisons covered a range of biomass from 5 to over 200 g dry wt.·m^{-2} (Table 2). In six of seven cases, no significant (Kruskal–Wallis test; $P < 0.01$) difference could be demonstrated among frequency distributions of estimated macrophyte biomass. This indicates that no quadrat size tends to yield higher biomass estimates than any other. The lone significant difference was found in Lake Massawippi where small quadrats yielded very low estimates of standing biomass. This effect was predicted by divers who noticed that smallest PVC quadrats, dropped at randomly chosen points, were not heavy enough to penetrate dense clumps of *M. spicatum* and tended to slide toward bare spaces between them. This effect could be avoided either by using heavier quadrats or by dropping very dense colored objects at randomly chosen points and orienting the quadrats to these weights in a predetermined fashion (e.g. corner, center, midside, etc.).

Precision

The major goal in designing a sampling program is to achieve an accurate measurement with high precision for the least effort. The size of the sampler or quadrat and the number of replicate samples taken can be varied so that the minimum number of samples of a given size may be extracted to yield a specified degree of precision. To date, the only means of calculating a priori the number of macrophyte samples needed to yield a biomass estimate with a given level of precision has been to assume that macrophyte biomass estimates follow the Poisson or negative binomial distribution. The actual variance function, or the relationship of the sample variance to the mean biomass and sampler size, has not previously been determined. In fact, variance functions for population variables other than counts of organisms have never been attempted (Taylor 1984).

To this end, we have collected 1200 published and unpublished observations of s^2 and $\bar{x}$ of dry weight biomass estimates. These data cover the range of habitats for which quantitative observations are available. Only data on true replicate samples (same date/same station) were included in the data set.[3] The range of estimated mean biomass is from 0.002 to 3700 g dry wt.·m^{-2}, the variance from 0.0001 to 1.8×10^6, sampler area from 100 to 10 000 cm^2, and n from 3 to 26. Data from marine systems are rare in the dataset (1%), and most data were collected by quadrat harvest (98%). When considered on a per

[3]The data are available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada K1A 0S2.

TABLE 3. Analysis of variance table for regression of $\log_{10} s^2$ as a function of $\log_{10} \bar{x}$ (mean macrophyte biomass, g dry wt. $\cdot$ mg^{-2}) and $\log_{10} A$ (sampler area, cm^2) (equations 1 and 3). F' (partial F-value) is calculated as the increase in model SS when each variable is entered into the multiple regression as the last variable. F' is a measure of the variation in $\log_{10} s^2$ accounted for by each independent variable. The probability of obtaining any of these F or F' values by chance is $\ll 0.001$.

Source of variation	df	SS	F	R^2	F'
Model (equation 1)	2	4255	11 610	0.95	
Error	1197	219			
Total	1199	4475			
Independent variable					
$\log_{10} \bar{x}$					23 027
$\log_{10} A$					37

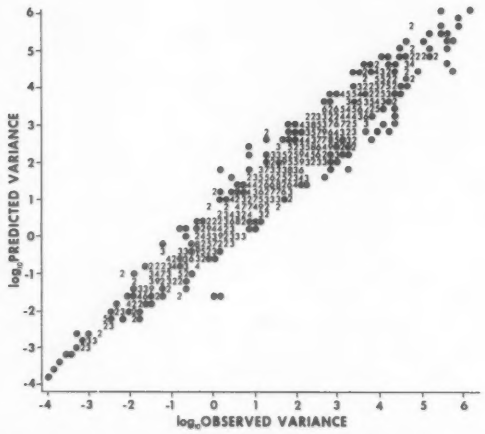


FIG. 3. Relationship between observed variance of replicate macrophyte biomass estimates (1200 sets of estimates) and the variance predicted from equation 4. Plotted digits indicate the number of observations at a given location.

square metre basis, macrophyte biomass appears spatially aggregated because $s^2 > \bar{x}$ in 83% of the observations collected.

Using multiple regression analysis, we found that s^2 of replicate macrophyte samples is predictable from the mean standing biomass ($\bar{x}$) and that a significant amount of residual variation in s^2 is accounted for by the sampler area (A) (Table 3). The regression equation ($r^2 = 0.95$) to predict the sampling variance of macrophyte biomass estimates is

$$(3) \quad \log s^2 = 0.759 + 1.567 (\log \bar{x}) - 0.157 (\log A)$$

or

$$(4) \quad s^2 = 5.75 \bar{x}^{1.567} A^{-0.157}.$$

Observed and predicted variances are very similar and highly correlated (Fig. 3). Other regression models were attempted, including models containing interaction terms, but this one proved to be the most accurate and precise. Neither the Poisson distribution nor the negative binomial with common k could yield as accurate predictions of spatial variation of macrophyte biomass (Fig. 4) because in the former case $s^2 = \bar{x}$, while in the latter $s^2 = \bar{x}^2/k + \bar{x}$ with a single k common to all samples. Tested on independent data, the Poisson distribution severely underestimated s and the negative binomial ($k = 2$) severely

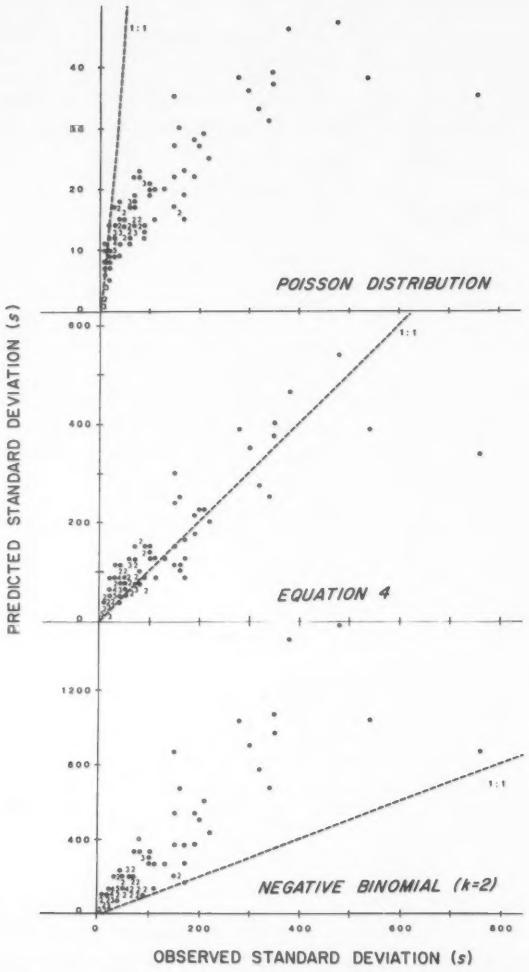


FIG. 4. Independent test of equation 4 for predicting sampling variability of macrophyte biomass estimates. Relationship is shown between observed standard deviations of macrophyte biomass estimates in studies not used for development of equation 4 (Center and Spencer 1981; Kimbel and Carpenter 1981; Ogden and Ogden 1982; Thommen and Westlake 1981; Van der Valk and Bliss 1971; Van der Valk and Davis 1980) and standard deviations predicted assuming the Poisson distribution, the negative binomial distribution (common k = 2), and equation 4. Independent observations of emergent and submergent biomass include data from lake, river, stream, and marine ecosystems. The broken line indicates a 1:1 correspondence between predictions and observations. Note unequal scales on vertical axes.

overestimated s, while equation 4 predicted standard deviations very close to those actually observed (Fig. 4).

Because different species of macrophytes possess differing modes of reproduction and dispersal, as well as differing ecological requirements, we might expect the biomass of aquatic macrophyte species to be differentially distributed in space. Analysis of the residuals should indicate the relative degree of spatial variability of the biomass of macrophyte species. Table 4 shows the residuals from equation 3 summed over a variety of taxonomic groups. The t-values indicate that many taxonomic groups have residuals that differ significantly from the overall

TABLE 4. Analysis of residuals from equation 3 by taxonomic group. Shown are the arithmetic mean and the variance of residuals in $\log_{10}$ form. n' is the number of sets of replicate samples of each taxon in the dataset, and t is the t-statistic comparing the mean residual of the subgroups (i.e. taxon) with the average of the residuals of all other samples assuming unequal variances (Prepas 1984, p. 298). r^2 is the coefficient of determination for the linear relationship between the residuals in $\log_{10}$ form and $\log_{10} \bar{x}$ for each taxon. $^*P < 0.05$; $^{**}P < 0.01$. Taxonomy follows the authors cited in Table 1.

Group	Mean	Variance	n'	t	df	r^2
Potamogeton praelongus	0.66	0.16	11	5.55**	10	0.34
Chara sp.	0.42	0.06	8	4.89**	7	0.40
Phragmites australis	0.35	0.04	3	3.05	2	0.07
Ranunculus sp.	0.34	0.07	8	3.71**	7	0.43
Cabomba caroliniana	0.26	0.05	15	4.36**	15	0.07
Potamogeton robbinsii	0.25	0.21	24	2.75*	24	0.20
Myriophyllum heterophyllum	0.22	0.15	4	1.15	3	0.32
Potamogeton richardsonii	0.22	0.06	4	1.81	3	0.46
Eriocaulon septangularis	0.21	0.11	8	1.81	7	0.43
Potamogeton crispus	0.20	0.11	14	2.26*	13	0.39*
Isoetes sp.	0.20	0.13	11	1.84*	10	0.01
Nitella hyalina	0.19	0.10	18	2.61**	18	0.15
Ceratophyllum demersum	0.19	0.06	5	1.73	4	0.21
Myriophyllum spicatum	0.19	0.13	103	5.56**	132	0.22**
Heteranthera dubia	0.18	0.09	23	2.95**	24	0.01
Ruppia maritima	0.17	0.14	11	1.51	10	0.05
Potamogeton cheesemannii	0.12	0.03	7	1.74*	6	0.01
Chara globularis	0.12	0.24	80	2.24*	88	0.06
Potamogeton gramineus	0.10	0.15	26	1.33	26	0.30**
Ranunculus bandooli	0.10	0.17	24	1.15	24	0.71**
Najas sp.	0.09	0.07	22	1.57	23	0.17
Myriophyllum triphyllum	0.09	0.05	8	1.14	7	0.23
Vallisneria americana	0.07	0.07	32	1.47	35	0.27**
Elodea canadensis	0.03	0.17	46	0.58	49	0.03
Nitella sp.	−0.03	0.07	17	−0.49	17	0.05
M. spicatum/monospecific	−0.05	0.46	17	−0.34	16	0.07
Chara coralina	−0.06	0.17	21	−0.63	21	0.01
Zannichellia marina	−0.07	0.01	3	−1.00	2	0.94
Chara baltica	−0.07	0.09	47	−1.52	54	0.02
Total biomass/mixed spp.	−0.07	0.20	258	−2.76**	391	0.01
Potamogeton pectinatus	−0.16	0.16	119	−4.48**	150	0.01
Chara cannescens	−0.17	0.21	35	−2.21*	36	0.08
Tolypella sp.	−0.19	0.08	21	−3.21**	22	0.26*
Zannichellia sp.	−0.20	0.09	14	−2.49*	14	0.06
Ruppia cirrhosa	−0.27	0.15	91	−7.02**	111	0.05*
Eichhornia crassipes	−0.42	0.10	12	−4.59**	11	0.06
Zostera capricorni	−0.45	0.06	8	−5.31**	7	0.01

mean residual. These analyses suggest that the biomass of the large macrophyte *P. praelongus* is more highly variable, while the biomass of dense floating plants such as *Eichhornia crassipes* and marine macrophytes such as *Zostera capricorni* are less variable than average. Nonparametric analyses of the residuals also suggest that the variance of marine macrophyte biomass is overestimated by equation 4 (Kruskal– Wallis test; $P < 0.01$). On the other hand, equation 4 works equally well for macrophytes growing in lakes, rivers, and streams (Kruskal–Wallis test; $P = 0.79$). In addition, data collected using quadrats and other collection methods (e.g. rakes, rotary cutters, grabs) appear to follow the same relationship (Mann–Whitney test; $P = 0.08$). Equation 4 works well for both aboveground and total biomass estimates (Mann–Whitney test; $P = 0.19$).

The last column of Table 4 shows the coefficient of determination (r^2) for the linear relation between the residuals from equation 3 (in $\log_{10}$ form) and the logarithm of mean standing biomass ($\bar{x}$). This analysis determines whether the overall regression coefficient for the effect of $\bar{x}$ (i.e. b in equation 1) departs significantly from that seen within each taxonomic group. There are some significant relationships, but only one accounts for >50% of the variation in the residuals (*Ranunculus bandooli*). This analysis suggests that a few species of macrophytes increase their spatial variation in biomass more rapidly with increased biomass than other species. Graphical analysis (Draper and Smith 1981) revealed no nonlinear trends in the residuals or lack of fit of various species with respect to the effect of A.

Requisite Sample Number

Equation 4 can be used to calculate the number of replicate quadrat samples (n) that one must take to yield a given level of precision. The predicted variance can be calculated by rearranging equation 1 (analogous to equation 4):

$$(5) \quad s^2 = a\bar{x}^b A^c$$

where all variables are as in equation 1. Because the standard

TABLE 5. Number of replicate samples needed for various sampler sizes and levels of aquatic macrophyte biomass in order that the SE of replicate samples average 20% of the mean standing biomass. Calculations are from equation 10.

Sampler size (cm^2)	Macrophyte biomass (g dry wt.·m^{-2})				
	3.2	10	32	100	320
100	42	26	16	9	6
316	35	21	13	8	5
1 000	29	18	11	7	4
3 162	25	15	9	6	3
10 000	21	12	8	5	3

error (SE) is calculated

$$(6) \quad \text{SE} = \sqrt{s^2/n} = (s^2)^{1/2} n^{-1/2}$$

the standard error can be approximated:

$$(7) \quad \text{SE} = a^{1/2} \bar{x}^{b/2} A^{c/2} n^{-1/2}.$$

If we desire a level of precision that is approximately 20% of the mean ($p = 0.2$ and $\text{SE} = 0.2\bar{x}$), then the number of replicate quadrat samples needed can be calculated by rearranging equation 7. In the general case $\text{SE} = p\bar{x}$ and substituting into equation 7:

$$(8) \quad p\bar{x} = a^{1/2} \bar{x}^{b/2} A^{c/2} n^{-1/2}.$$

By rearranging equation 8 and combining terms, we find that

$$(9) \quad \hat{n} = a\bar{x}^{b-2} A^c p^{-2}$$

where $\hat{n}$ is the predicted number of samples needed to obtain a level of precision such that the standard error equals $p\bar{x}$.

For aquatic macrophytes in particular, we substitute the coefficients from equation 4 into equation 9 to yield

$$(10) \quad \hat{n} = 5.75 \bar{x}^{-0.433} A^{-0.157} p^{-2}.$$

This equation shows that the requisite number of replicate quadrat samples (Table 5) decreases with increased standing macrophyte biomass, increased quadrat area, and decreased precision (i.e. increased p). It follows from Fig. 4 and equation 10 that assumption of the Poisson distribution for macrophyte biomass estimates would result in a lack of statistical precision, while assumption of the negative binomial distribution would result in wasted sampling effort.

The predictions of $\hat{n}$ from equation 10 (Table 5) are made for the average of all types of macrophytes for which data were available. Table 4 shows that the biomasses of some taxa differ significantly from the overall variance relation shown in equation 4. We do not know whether these differences among species will be found consistently in all habitats. The available evidence suggests that it would be prudent to take more samples than predicted by equation 10 if a significantly positive t is shown in column 4 of Table 4. Correction for such departures can be made by multiplication of $\hat{n}$ by the antilog (base 10) of the mean residual (column 1 of Table 4). For *Phragmites australis*, $10^{0.35} = 2.24$, and therefore the data in Table 4 should be multiplied by 2.24 to yield more conservative estimates of $\hat{n}$ for this species. Where r^2 in column 6 of Table 4 is statistically significant, $\hat{n}$ might be better calculated as

$$(11) \quad \hat{n} = af\bar{x}^{b+g-2} A^c p^{-2}$$

TABLE 6. Regression coefficients for taxa in which residual analysis (Table 4) showed a significant relationship ($P < 0.05$) between the residuals from equation 3 and $\log_{10} \bar{x}$. f and g are fitted constants such that residual (from equation 3) = $\log_{10} f + g(\log_{10} \bar{x})$. n' is the number of sets replicate observations of each taxon included in the dataset.

Species	n'	f	g
Potamogeton crispus	14	2.173	0.266
Myriophyllum spicatum	103	1.260	0.180
Potamogeton gramineus	26	1.989	0.241
Ranunculus bandooli	24	1.515	0.316
Vallisneria americana	32	1.791	−0.223
Tolypella spp.	21	1.056	0.220
Ruppia cirrhosa	91	0.570	−0.099

where values of f and g can be found in Table 6 and all other variables are as in equation 9. Although these corrections can be substantial in some cases (e.g. *P. praelongus*), equation 10 can probably be used without correction for most design purposes.

Cost Efficiency

Table 5 shows that there exist many combinations of quadrat size and sample number that can yield the same level of precision for a given biomass. Decisions as to which combination to use are almost entirely based on cost (Cochran 1977, p. 84). In the estimation of total macrophyte biomass, most of the effort and cost are expended in sampling the macrophytes. We found that the time required for a diver to collect a macrophyte sample was an increasing, decelerating function of quadrat size (Fig. 5A) but was relatively insensitive to the mass macrophyte collected (Fig. 5B). Collection time was poorly fitted as an exponential or power function of quadrat size because this relationship is asymptotic. Divers spend less time per unit bottom area as quadrat area increases. The trend in the data is approximated well ($r^2 > 0.54$) by the asymptotic function

$$(12) \quad T = 39.95(1 - e^{-0.00539A})$$

where T is collection time (minutes), A is the quadrat size (square centimetres), and e is the base of natural logarithms. The large amount of scatter in Fig. 5A is due primarily to differences among divers. For example, one inexperienced diver took an average of 16 min longer to collect the macrophytes from a 1-m^2 quadrat than a highly experienced diver, although no differences in biomass collected were found. The shape of equation 12 is accurate for a diver of average experience, and it provides predictions of average sampling cost for our aquatic macrophyte biomass estimates.

The average cost of different combinations of quadrat size and sample number can be approximated by multiplication of equations 10 and 12 (Table 7). The amount of effort needed to collect macrophyte samples yielding a given precision decreases with standing biomass and increases rapidly with size of quadrat employed. For a given precision, large quadrats (e.g. 1 m^2) require over 30 times the sampling effort of small quadrats (e.g. 100 cm^2). This finding would be accentuated if samples were to be sorted to species, because 100-cm^2 quadrat samples could often be sorted in <1 min, while 1-m^2 samples took several hours (M. R. Anderson and J. A. Downing, unpubl.). Other advantages of small quadrats are that they yield samples that are easier to store and transport, destroy less habitat, and are easier to ash (Wetzel 1965). It has long been suggested that small

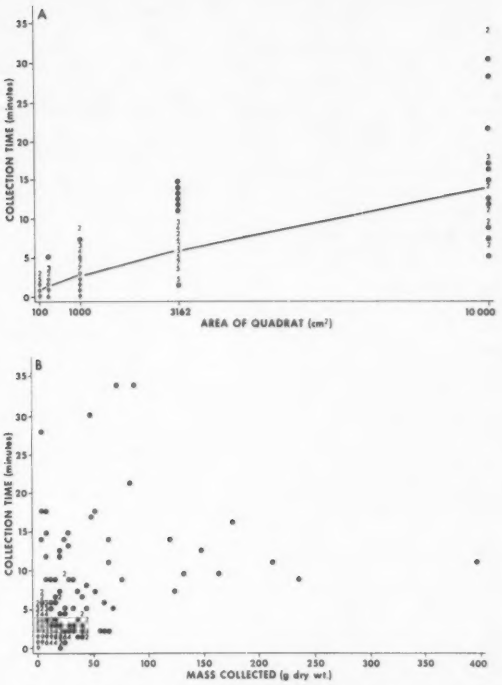


FIG. 5. (A) Asymptotic relationship between the amount of time divers took to collect macrophyte samples and the size of quadrat cleared. Median times are connected with a solid line. (B) Lack of relation between the collection time and the dry mass of macrophytes collected.

TABLE 7. Average amount of time (min) needed for macrophyte harvest for various sampler sizes and levels of aquatic macrophyte biomass in order that the SE of replicate samples average 20% of the mean standing biomass. Calculations were made by multiplication of equations 10 and 12.

Sampler size (cm^2)	Macrophyte biomass (g dry wt.·m^{-2})				
	3.2	10	32	100	320
100	9	6	3	2	1
316	24	14	9	5	3
1 000	61	38	23	15	8
3 162	157	94	56	38	19
10 000	350	200	133	83	50

samples yield efficient estimates of biomass and populations of other organisms (Downing 1979; Green 1979; Hanson 1930, 1934; Hasel 1938; Hendricks 1956; Justesen 1932; Pechanec and Stewart 1940; Smith 1938; Ursic and McClurkin 1959). Pringle (1984) found this to be true for sampling Irish moss off western Prince Edward Island. We found small samples to be the most efficient for macrophyte biomass research, in general.

Recommendations

The use of quadrat sampling by SCUBA avoids the possible losses using remote samplers. As long as randomization is complete, all quadrat sizes yield the same estimates of standing biomass (Table 2). Caution should be exercised in the placement of small quadrats, however, especially in dense macrophyte beds.

Analysis of the spatial variance of aquatic macrophyte biomass shows that greater replication must be used when sampling macrophyte beds of low standing biomass or when using small quadrats. The use of small quadrats can double the number of replicate samples required for a given level of precision (equation 10; Table 5). Analysis of the cost of sampling macrophyte biomass shows, however, that the use of large numbers of small quadrats can result in 30-fold savings in sampling time (Table 7). Comparison with the sampling habits of aquatic ecologists (Fig. 1 and 2) and the recommendations of handbooks and methods manuals (e.g. Lind 1979; Schwoerbel 1970; Welch 1948; Wetzel and Likens 1979; Wood 1975) suggests that many ecologists could save sampling effort or improve levels of precision by using smaller quadrats. The number of requisite samples should be estimated in advance, based on the sampling experiences of other ecologists (equation 10). Equation 10 can be applied by guessing the macrophyte biomass to be encountered in the field, or by inspection of published tables of macrophyte biomass data (e.g. Sculthorpe 1967; Wetzel 1983). In addition, equations 3 and 10 simplify preliminary sampling because they provide guidance on choice of quadrat size, and require that the preliminary sample only provide an estimate of $\bar{x}$. The use of large numbers of small-sized samples can result in much less costly macrophyte biomass estimates with high levels of precision.

The use of small quadrats results not only in reduced sampling cost but can also result in skewed biomass frequency distributions (Cain and Evans 1952; Evans 1952; Pechanec and Stewart 1940). Skewed frequency distributions can lead to bias in analyses based on the normal distribution (Scheffé 1959). Data must be properly transformed to stabilize the variance, as this will often alleviate skewness and increase the probability of additivity (Bliss and Owen 1958; Snedecor and Cochran 1967; Southwood 1966; Tukey 1968). The fourth-root transformation should perform well for most macrophyte data because the general b in equation 1 is 1.57 (equation 4) (Downing 1979; Taylor 1961). In some cases, transformation to stabilize the variance will not be adequate. The performance of transformation should always be verified, as improper transformation can lead to inferential bias. When there is doubt, parallel analyses should be performed using nonparametric methods.

Acknowledgments

Financial support for this study was provided through grants from the Canadian National Sportsmen's Fund (grants 5-R33, 6-R52), a NSERC operating grant to JAD, and a team grant (Groupe d'Ecologie des Eaux Douces, l'Université de Montréal) from the Quebec Minister of Education (FCAR). We thank "the army": H. Cyr, L. Glennie, P. Chambers, M. Portier, S. Kalff, H. L'Heureux, D. Brumelis, and M. Stewart for tireless and patient field work. Finally, we thank C. Howard-Williams, T. Kiørboe, N. Thyssen, S. A. Shepherd, C. Menzie, H. Kirkman, D. Landers, E. B. Welch, S. Engel, M. G. Kelly, P. H. Rich, J. W. Barko, and R. B. Sheldon for their generosity, G. Pageau and three anonymous reviewers for criticisms, and all who answered our letters with enthusiasm.

References

BAYLEY, S., V. D. STOTTS, P. F. SPRINGER, AND J. STEENIS. 1978. Changes in submerged aquatic macrophyte populations at the head of Chesapeake Bay 1958–1975. Estuaries 1: 171–182.

BLISS, C. I., AND A. R. G. OWEN. 1958. Negative binomial distributions with a common *k*. Biometrika 45: 37–58.

CAIN, S. A., AND F. C. EVANS. 1952. The distribution patterns of three plant species in an old field community in southeastern Michigan. Contrib. Lab. Vertebr. Biol. Univ. Mich. No. 52: 11 p.

CARIGNAN, R., AND J. KALFF. 1982. Phosphorus release by submerged macrophytes: significance to epiphyton and phytoplankton. Limnol. Oceanogr. 27: 419–427.

CATTANEO, A. 1983. Grazing on epiphytes. Limnol. Oceanogr. 28: 124–132.

CENTER, T. D., AND N. R. SPENCER. 1981. The phenology and growth of water hyacinthe (*Eichhornia crassipes*) ((Mart.) Solms) in a eutrophic north central Florida lake. Aquat. Bot. 10: 1–32.

COCHRAN, W. G. 1977. Sampling techniques. Wiley, New York, NY.

CONOVER, W. J. 1971. Practical non-parametric statistics. Wiley, New York, NY.

DAVIES, G. S. 1970. Productivity of macrophytes in Marion Lake, British Columbia. J. Fish. Res. Board Can. 27: 71–81.

DOWNING, J. A. 1979. Aggregation, transformation, and the design of benthos sampling programs. J. Fish. Res. Board Can. 36: 1454–1463.

1984. Sampling the benthos of standing waters, p. 87–130. *In* J. A. Downing and F. H. Rigler [ed.] A manual of methods for the assessment of secondary productivity in fresh waters. IBP Handbook No. 17. 2nd ed. Blackwell Scientific Publications, Oxford.

DOWNING, J. A., AND H. CYR. 1985. Quantitative estimation of epiphytic invertebrate populations. Can. J. Fish. Aquat. Sci. 42: 1570–1579.

DRAPER, N. R., AND H. SMITH. 1981. Applied regression analysis. Wiley, New York, NY. 709 p.

ELLIOTT, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Sci. Publ. 25. 2nd ed. Freshwater Biological Association, Cumbria, England. 160 p.

ENGEL, S. 1982. Evaluating sediment blankets and a screen for macrophyte control in lakes. Final Report, Office of Inland Lake Renewal, DNR, Madison, WI.

EVANS, F. C. 1952. The influence of size of quadrat on the distributional patterns of plant populations. Contrib. Lab. Vertebr. Biol. Univ. Mich. No. 54.

FASSETT, N. C. 1966. A manual of aquatic plants. University of Wisconsin Press, Madison, WI. 405 p.

GEORGE, E. L., AND W. F. HADLEY. 1979. Food habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieui*) young of the year. Trans. Am. Fish. Soc. 108: 253–261.

GRACE, J. B., AND L. J. TILLEY. 1976. Distribution and abundance of submerged macrophytes, including *Myriophyllum spicatum* L. (Angiospermae), in a reactor cooling reservoir. Arch. Hydrobiol. 77: 475–487.

GREEN, R. H. 1979. Sampling design and statistical methods for environmental biologists. Wiley, New York, NY. 257 p.

HANSON, H. C. 1930. Size of list quadrat for use in determining effects of different systems of grazing upon *Agropyron smithii* mixed prairie. J. Agric. Res. 41: 549–560.

1934. A comparison of methods of botanical analysis of the native prairie in western North Dakota. J. Agric. Res. 49: 815–842.

HASEL, A. A. 1938. Sampling error in timber surveys. J. Agric. Res. 57: 713–736.

HENDRICKS, W. A. 1956. The mathematical theory of sampling. The Scarecrow Press, New Brunswick, NJ. 364 p.

HOWARD-WILLIAMS, C. 1978. Growth and production of aquatic macrophytes in a south-temperate saline lake. Verh. Int. Ver. Limnol. 20: 1153–1158.

1980. Aquatic macrophyte communities of the Wilderness Lakes: community structure and associated environmental conditions. J. Limnol. Soc. South. Afr. 6: 85–92.

HOWARD-WILLIAMS, C., AND B. R. ALLANSON. 1978. The limnology of Swartvlei with special reference to production and nutrient dynamics in the littoral zone. Rhodes Univ. Inst. Freshwater Stud. Spec. Rep. 78/3. Grahamstown, South Africa. 280 p.

1981. An integrated study on littoral and pelagic primary production in a Southern African coastal lake. Arch. Hydrobiol. 92: 507–534.

HOWARD-WILLIAMS, C., J. DAVIS, AND S. PICKMERE. 1982. The dynamics of growth, the effects of changing area and nitrate uptake by watercress *Nasturtium officinale* R. Br. in a New Zealand stream. J. Appl. Ecol. 19: 589–601.

HOWARD-WILLIAMS, C., AND G. M. LENTON. 1975. The role of the littoral zone in the functioning of a shallow tropical lake ecosystem. Freshwater Biol. 5: 445–459.

HOWARD-WILLIAMS, C., AND M. R. M. LIPTROT. 1980. Submerged macrophyte communities in a brackish South African estuarine-lake ecosystem. Aquat. Bot. 9: 101–116.

JACOBY, J. M., D. D. LYNCH, E. B. WELCH, AND M. A. PERKINS. 1982. Internal phosphorus loading in a shallow eutrophic lake. Water Res. 16: 911–919.

JOHN, D. M., D. LIEBERMAN, M. LIEBERMAN, AND M. D. SWAINE. 1980. Strategies of data collection and analysis of subtidal vegetation, chap. 10, p. 265–284. *In* J. H. Price, D. E. G. Irvine, and W. F. Farnham [ed.] The shore environment. Vol. 1: Methods. Syst. Assoc. Spec. Vol. No. 17. Academic Press, New York, NY.

JUSTESEN, S. H. 1932. Influence of size and shape of plots on the precision of field experiments with potatoes. J. Agric. Sci. 22: 365–372.

KIMBEL, J. C., AND S. R. CARPENTER. 1981. Effects of mechanical harvesting on *Myriophyllum spicatum* L. regrowth and carbohydrate allocation to roots and shoots. Aquat. Bot. 11: 121–127.

KIRKMAN, H., I. H. COOK, AND D. D. REID. 1982. Biomass and growth of *Zostera capricorni* ASCHERS in Port Hocking, NSW, Australia. Aquat. Bot. 12: 57–67.

LANDERS, D. H. 1982. Effects of naturally senescing aquatic macrophytes on nutrient chemistry and chlorophyll *a* of surrounding waters. Limnol. Oceanogr. 27: 428–439.

LAUGHLIN, D. R., AND E. E. WERNER. 1980. Resource partitioning in two coexisting sunfish: pumpkinseed (*Lepomis gibbosus*) and northern longear sunfish (*Lepomis megalotis peltastes*). Can. J. Fish. Aquat. Sci. 37: 1411–1420.

LIND, O. T. 1979. Handbook of common methods in limnology. C. V. Mosby Co., London. 154 p.

MARQUARDT, D. 1963. An algorithm for least-squares estimation of nonlinear parameters. J. Soc. Ind. Appl. Math. 11: 431–441.

MENZIE, C. A. 1979. Growth of aquatic plant *Myriophyllum spicatum* in a littoral area of the Hudson River estuary. Aquat. Bot. 6: 365–375.

NICHOLSON, S. A., AND B. AROYO. 1973. Macrophyte zonation in an undisturbed bay, p. 285–350. Chatauqua Lake Studies 1b. Fredonia, NY.

NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, AND D. H. BENT. 1975. SPSS: statistical package for the social sciences. McGraw-Hill, New York, NY. 675 p.

OGDEN, J. C., AND N. B. OGDEN. 1982. A preliminary study of two representative seagrass communities in Palau, West Caroline Islands (Micronesia). Aquat. Bot. 12: 229–244.

PECHANEC, J. F., AND G. STEWART. 1940. Sagebrush-grass range sampling studies: size and structure of sampling unit. J. Am. Soc. Agron. 32: 669–682.

PREPAS, E. E. 1984. Some statistical methods for the design of experiments and analysis of samples, p. 266–335. *In* J. A. Downing and F. H. Rigler [ed.] A manual of methods for the assessment of secondary productivity in fresh waters. IBP Handbook No. 17. 2nd ed. Blackwell Scientific Publications, Oxford.

PRINGLE, J. D. 1984. Efficiency estimates for various quadrat sizes used in benthic sampling. Can. J. Fish. Aquat. Sci. 41: 1485–1489.

RICH, P. H., R. G. WETZEL, AND N. V. THUY. 1971. Distribution of production and role of aquatic macrophytes in a southern Michigan marl lake. Freshwater Biol. 1: 3–21.

ROBINSON, B. 1977. SPSS subprogram NONLINEAR–nonlinear regression. Manual No. 477, Vogelman Computing Center, Northwestern University, Evanston, IL. 27 p.

SAHAI, R., AND A. B. SINHA. 1970. Contribution to the ecology of Indian aquatics. I. Seasonal changes in biomass of water hyacinthe (*Eichhornia crassipes* (Mart.) Solms.). Hydrobiologia 35: 376–382.

SCHEFFÉ, H. 1959. The analysis of variance. Wiley, New York, NY. 477 p.

SCHWOERBEL, J. 1970. Methods of hydrobiology. Pergamon Press, London. 200 p.

SCULTHORPE, C. D. 1967. The biology of aquatic vascular plants. St. Martin's Press, New York, NY. 610 p.

SHELDON, R. B., AND C. W. BOYLEN. 1978. An underwater survey method for estimating submerged macrophyte population density and biomass. Aquat. Bot. 4: 65–72.

SMITH, H. F. 1938. An empirical law describing heterogeneity in the yields of agricultural crops. J. Agric. Sci. 28: 1–23.

SNEDECOR, G. W., AND W. C. COCHRAN. 1967. Statistical methods. The Iowa State University Press, Ames, IA. 593 p.

SOUTHWOOD, T. R. E. 1966. Ecological methods with particular reference to the study of insect populations. Chapman and Hall, London. 391 p.

SUKHATME, P. V. 1947. The problem of plot size in large scale yield surveys. J. Am. Stat. Soc. 42: 297–310.

TAYLOR, L. R. 1961. Aggregation, variance and the mean. Nature (Lond.) 189: 732–735.

1984. Assessing and interpreting the spatial distributions of insect populations. Annu. Rev. Entomol. 29: 321–357.

THOMMEN, G. H., AND D. F. WESTLAKE. 1981. Chalk streams in Dorset, England. Aquat. Bot. 11: 21–36.

TUKEY, J. W. 1968. The true purpose of transformation. Biometrics 24: 1041.

UNNI, K. S. 1977. The distribution and production of macrophytes in Lunz Mittersee and Lunz Untersee. Hydrobiologia 56: 89–94.

URSIC, S. J., AND D. C. MCCLURKIN. 1959. Small plots for measuring vegetation composition and cover, p. 70–78. *In* Techniques and methods of measuring understory vegetation. Proceedings of a symposium at Tifton, Georgia, Oct. 1958. Southern Forest Experiment Station and Southeastern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Washington, DC.

VAN DER VALK, A. G., AND L. C. BLISS. 1971. Hydrarch succession and net primary production of oxbow lakes in central Alberta. Can. J. Bot. 49: 1177–1199.

VAN DER VALK, A. G., AND C. B. DAVIS. 1980. The impact of a natural drawdown on the growth of four emergent species in a prairie glacial marsh. Aquat. Bot. 9: 301–321.

VAN DYNE, G. M., W. G. VOGEL, AND H. G. FISSER. 1963. Influence of small plot size and shape on range herbage production estimates. Ecology 44: 746–759.

WELCH, E. B., J. B. MICHAUD, AND M. A. PERKINS. 1982. Alum control of internal phosphorus loading in a shallow lake. Water Resour. Bull. 18: 929–936.

WELCH, P. S. 1948. Limnological methods. McGraw-Hill Inc., New York, NY. 381 p.

WESTLAKE, D. F. 1965. Some basic data for investigations of the productivity of aquatic macrophytes. Mem. Ist. Ital. Idrobiol. 18 (suppl.): 229–248.

1969. Macrophytes, p. 25–32, 103–107. *In* R. A. Vollenweider [ed.] A manual on methods for measuring primary production in aquatic environments. IBP Handbook No. 12. Blackwell Scientific Publications, Oxford.

WETZEL, R. G. 1965. Techniques and problems of primary productivity measurements in higher aquatic plants and periphyton. Mem. Ist. Ital. Idrobiol. 18 (suppl.): 249–267.

1983. Limnology. Saunders College Publ., Philadelphia, PA. 767 p.

WETZEL, R. G., AND G. E. LIKENS. 1979. Limnological analyses. W. B. Saunders Co., London. 357 p.

WIEGERT, R. G. 1962. The selection of an optimum quadrat size for sampling the standing crop of grasses and forbs. Ecology 43: 125–129.

WONG, S. L., AND B. CLARK. 1979. The determination of desirable and nuisance plant levels in streams. Hydrobiologia 63: 223–230.

WOOD, R. D. 1975. Hydrobotanical methods. University Park Press, Baltimore, MD. 173 p.

Interactive Effects of Cadmium, Polychlorinated Biphenyls, and Fuel Oil on Experimentally Exposed English Sole (*Parophrys vetulus*)

Linda Rhodes and Edmundo Casillas

Northwest and Alaska Fisheries Center, Environmental Conservation Division, National Marine Fisheries Service, 2725 Montlake Boulevard East, Seattle, WA 98112, USA

Barbara McKnight

Department of Biostatistics, University of Washington, Seattle, WA 98195, USA

and William Gronlund, Mark Myers, O. Paul Olson, and Bruce McCain

Northwest and Alaska Fisheries Center, Environmental Conservation Division, National Marine Fisheries Service, 2725 Montlake Boulevard East, Seattle, WA 98112, USA

Rhodes, L., E. Casillas, B. McKnight, W. Gronlund, M. Myers, O. P. Olson, and B. McCain. 1985. Interactive effects of cadmium, polychlorinated biphenyls, and fuel oil on experimentally exposed English sole (*Parophrys vetulus*). Can. J. Fish. Aquat. Sci. 42: 1870–1880.

Investigation of the interactive effects of representatives of three classes of compounds was performed, using English sole (*Parophrys vetulus*) as the test organism. Juvenile fish were exposed orally to cadmium chloride and Aroclor 1254 (PCB), either independently or simultaneously, for a 4-wk period, followed by exposure to seawater-accommodated No. 2 diesel fuel for 2 wk. Blood was collected for analysis of serum constituents, and tissue samples were examined for histological changes. Hepatocellular necrosis, regeneration, and karyomegaly were observed. Differential lesion prevalences were observed among the exposure groups, with high proportions among cadmium-exposed fish, low proportions among PCB-exposed fish, and intermediate proportions in combination cadmium- plus PCB-exposed fish. Levels of aspartate aminotransferase activity and magnesium in the sera of these groups exhibited similar patterns. Depressed serum calcium levels in both PCB-exposed and cadmium- plus PCB-exposed groups were found after the first week, and lower serum albumin concentrations occurred in all cadmium- and PCB-exposed groups following the third week. Subsequent exposure to No. 2 diesel fuel produced few effects in any of the exposure groups. The observed antagonistic effect of Aroclor 1254 against cadmium toxicity emphasizes the importance of employing multiple as well as single contaminant exposure in toxicity studies.

Les auteurs ont étudié les effets de l'interaction de trois composés sur la sole anglaise (*Parophrys vetulus*). Des juvéniles ont été exposés à des solutions de chlorure de cadmium et d'Aroclor 1254 (BPC) soit séparément ou simultanément, pendant 4 sem, puis à du gasoil nº 2 adapté à l'eau salée pendant 2 sem. On a recueilli des échantillons de sang et de tissus pour l'analyse respective des composantes sériques et des changements histologiques, ce qui a permis d'observer une nécrose hépatocellulaire, une regénération et une caryomégalie, ainsi que des lésions distinctives chez les groupes exposés. Ces lésions étaient nombreuses chez les poissons exposés au cadmium, peu nombreuses chez ceux exposés au BPC et moyennement nombreuses chez les individus exposés à un mélange de cadmium et de BPC. Les niveaux d'activité de l'aspartate aminotransférase et de magnésium dans le sérum de ces groupes étaient semblables. Des taux diminués de calcium sérique ont été observés chez les groupes exposés au BPC et au cadmium mélange au BPC après la première semaine; de plus faibles concentrations d'albumine sérique ont aussi été notées chez tous les groupes exposés au cadmium et au BPC après la troisième semaine. L'exploitation ultérieure à du gasoil nº a eu peu d'incidence chez tous les groupes exposés. L'effet antagoniste de l'Aroclor 1254 sur la toxicité du cadmium souligne l'importance d'utiliser des expositions multiples et simples dans les études sur la toxicité.

Received February 6, 1985
Accepted July 31, 1985
(J8107)

Reçu le 6 février 1985
Accepté le 31 juillet 1985

Laboratory studies of the effects of chemical pollution on fish have commonly involved exposure to individual chemicals (Chapman and Stevens 1978; Leatherland and Sonstegard 1978; Hawkins et al. 1980; Kiceniuk et al. 1980; Freeman et al. 1982). Since xenobiotic exposure in the environment very likely involves a multitude of toxicants whose potential interactions (e.g. synergistic, antagonistic, additive, etc.) are unknown, laboratory studies of the effects of multiple

TABLE 1. Numbers of animals in exposure and sampling (parentheses) regime. English sole were exposed weekly to cadmium or PCB or both. At 1 mo, one half of each group was exposed to seawater-accommodated No. 2 diesel fuel (indicated by an asterisk).

	Day of experiment									
	1	7	8	14	15	21	22	28	29	42
Untreated control	70	(10)	60	(10)	50	(10)	40	(10)	15 15*	(10) (10)*
Sham-fed control	70	(10)	60	(10)	50	(10)	40	(10)	15 15*	(10) (10)*
Cadmium exposed	70	(10)	60	(10)	50	(10)	39	(10)	14 11*	(10) (4)*
PCB exposed	70	(10)	60	(10)	50	(10)	40	(10)	15 15*	(10) (10)*
Cadmium plus PCB exposed	70	(10)	60	(10)	50	(10)	38	(10)	12 14*	(3) (10)*

contaminant exposure are important (Fingerman 1980; Hawkes 1980; Singh and Sivalingam 1982; Voyer and Heltshe 1984). Information obtained in this manner may contribute substantially to understanding environmental effects of contaminants on the health of marine biota. For this investigation, English sole (*Parophrys vetulus*) were exposed to representatives of three major classes of pollutants found in Puget Sound, Washington (Malins et al. 1984): a heavy metal, cadmium; a polychlorinated biphenyl (PCB), Aroclor 1254; and an aromatic hydrocarbon complex, No. 2 diesel fuel. Fish were exposed to these chemicals individually and in combination. This study specifically addressed the potential interactive effects on two sensitive indicators of health status of English sole, serum chemistry (Casillas et al. 1983) and organ histology (Malins et al. 1984, 1985), of initial exposure to cadmium and PCB followed by exposure to diesel fuel. The latter portion of this study was designed to address the question of whether prior exposure to a toxicant enhanced the expression of toxic effects upon subsequent exposure to a different toxicant.

Methods and Materials

Juvenile English sole (0+ age class) of an average total length of 102 mm (range 81–128 mm) and average weight of 9.3 g (range 4.4–20.6 g) were collected by otter trawl offshore of Meadow Point in Puget Sound, Washington, and were held for 2 wk in tanks receiving flow-through ambient seawater from Puget Sound at the National Marine Fisheries Service Field Laboratory at Mukilteo. Fish were fed to satiation daily on a mixture of chopped clams and euphausids through the holding and experimental periods, except for a 24-h period before each sampling.

The experiment was conducted over a subsequent 6-wk period. Animals were randomly distributed among 10 identical 216-L glass tanks (bottom surface area of 1.1 m^2) with a resulting initial density of 35 animals per tank. Tanks received ambient seawater from Puget Sound with water temperatures ranging from 8.5 to 10.5°C and salinities ranging from 20 to 26‰ during the experimental period. Flow rates were adjusted to 3 L/min.

The exposure and sampling regime is given in Table 1. Exposure conditions were designed to simulate dietary exposure to metal and chlorinated hydrocarbon contaminants followed by acute water borne exposure to a petroleum hydrocarbon mixture. Cadmium, a contaminant of known acute and chronic toxicity to marine organisms, has been found to be present in potential English sole food sources from Puget Sound, such as polychaete worms and clams (Malins et al. 1980). Concentrations of PCBs in potential food organisms and the stomach contents of English sole (Malins et al. 1984, 1985) have been found to exceed values found in the sediment. Since contamination of the diet with xenobiotics represents a significant, and often overlooked, route of exposure to English sole, gastric intubation was selected for administration of cadmium and PCB. Both compounds were dissolved in a molten agar medium to a standard concentration, and dosages were adjusted volumetrically to the weight of each fish. Intubation was performed using a 0.1-mL glass pipet cleaned with aqua regia and methylene chloride to introduce the dose directly into the stomach.

The oral exposure to cadmium and/or PCB constituted the first part of the experiment. Seventy English sole per group were exposed to one of the following treatments via gastric intubation: (1) 5 μg $CdCl_2$/g body wt, (2) 25 μg PCB (Aroclor 1254)/g body wt, (3) 5 μg $CdCl_2$ + 25 μg PCB/g body wt, (4) uncontaminated agar vehicle (sham-fed control), and (5) untreated sole (untreated control). Fish were intubated at weekly intervals for a 4-wk exposure period.

Number 2 diesel fuel, a commonly used fuel in boats, is frequently spilled into marine waters in varying quantities. Because of its widespread presence in commercial and pleasure boating activities, spills can occur on nearly all navigable waters. The second part of the experiment, which consisted of exposing half of the remaining fish in each of the five groups to a 2 mg/L seawater-accommodated fraction of No. 2 diesel fuel, simulated exposure to a diesel fuel spill. The fraction was administered using the solubilizing system described by Maynard and Weber (1981). The remaining half of each group was held in seawater only during this period to serve as respective controls.

Fifteen fish were sampled at the start of the experiment to serve as day 0 controls. Thereafter, 10 fish were sampled on a weekly basis (Table 1) except for the fifth week of the experiment when no samples were taken.

For each animal, a blood sample for serum analysis was collected by caudal peduncle excision. Blood samples were allowed to clot for 45 min at room temperature. The samples were then centrifuged for 15 min at 1500 × *g* at 4°C, and the serum separated. Serum samples were subsequently analyzed for aspartate aminotransferase activity (ASAT) and albumin, glucose, calcium, and magnesium concentrations using previously described procedures (Casillas et al. 1983).

Twelve organs were collected for histologic examination, including liver, kidney, small intestine, spleen, gonad, heart, gall bladder, brain, olfactory epithelium, gill, skin, and fin. Tissue samples were preserved in Dietrich's fixative (Gray 1954), embedded in paraffin, sectioned at 5 μm, and routinely stained with Mayer's hematoxylin and eosin–phloxine (Armed Forces Institute of Pathology 1968). Stains for specific structures or substances included Spicer's method for nucleic acids (Armed Forces Institute of Pathology 1968), periodic acid – Schiff's stain for glycogen and mucin (Preece 1972), phosphotungstic acid – hematoxylin for collagen (Preece 1972), and Gomori's iron reaction for hemosiderin (Preece 1972). Diagnoses were performed under a blind system, i.e. tank number, specimen number, and treatment information were not available to the slide readers until all diagnoses were completed.

All data were encoded for magnetic storage, and management and analyses of data were implemented using SPSS version 8.1 (Nie et al. 1975), Minitab version 3.1 (Ryan et al. 1981), and GLIM version 3.1 (Royal Statistical Society 1978).

Statistical Analyses

Analysis of mortality frequencies and serum chemistry results were made either by chi-square frequency distribution comparisons ($P \leq 0.05$) or by analysis of variance ($P \leq 0.05$) where appropriate.

Histopathology results were analyzed by Fisher's exact test and by the development of logistic regression models. Fisher's exact test was employed to test for significant differences in lesion prevalence between groups having a lesion prevalence of zero and groups with a nonzero lesion prevalence. When significant differences were found, only groups with nonzero lesion prevalences were further analyzed by regression modeling; otherwise, all groups were included in regression analysis.

Logistic regression models relating treatment status to the prevalence of various conditions was the next step. This type of model, used extensively to evaluate the relationship between potential risk factors and disease occurrence in studies of disease prevalences, is based on the natural logarithm of the odds of a condition; the logistic model has the general form

$$\log \frac{P(X)}{1 - P(X)} = \alpha + \sum_{k=1}^{K} \beta_k X_k.$$

In this formulation, $X = (X_1, X_2, \ldots, X_K)$ is a vector of variables, such as exposure to cadmium or exposure to PCB, which can indicate the presence or absence of a potential risk factor or the value of some continuous risk factor, and $P(X)$ is the probability of the condition of interest for an experimental subject with the particular combination of exposures to risk factors given by X.

Estimated coefficients β_k that were significantly ($P \leq 0.05$) different from zero provided strong evidence that the prevalence of the condition varied with different values of X_k. The models developed retained only those variables X_k that exhibited a significant effect on the value of $P(X)$.

Interpretation of the regression models included determination of the odds of lesion development associated with each risk factor. For exposure factors, the odds ratio measured the increased probability of lesion development relative to a baseline group of animals which depended upon the groups that were included in model development. When necessary, linear and quadratic time factors were employed in modeling. Odds ratios greater than 1 indicated increased probability of lesion development associated with the risk factor.

For a more thorough description of the methodology and application of these techniques see Breslow and Day (1980).

Results

Exposure to cadmium, either singly or in combination with other contaminants, resulted in significant mortalities ($P \leq 0.05$). No mortalities were observed among the control animals and only one mortality occurred among fish exposed to only PCB. Among the groups not exposed to fuel oil, significant mortality frequencies were observed among groups simultaneously exposed to cadmium and PCB (12 of 70 animals) and exposed to only cadmium (5 of 70 animals). Only one fuel-exposed group (cadmium only exposure) experienced mortalities (6 animals). Most of the deaths occurred during the fourth and fifth week of exposure, with a maximum of 50% in the cadmium- plus PCB-exposed group. Additionally, there were no significant differences in feeding activity among the experimental groups. Emaciation and other signs of nutritional deficiency were not apparent among the mortalities.

Histopathology

Of the organs examined microscopically for effects of xenobiotic exposure, only the liver and small intestine had lesions. In the liver, three abnormalities that were observed were necrosis, regeneration, and karyomegaly. These conditons affected only the hepatocytes; the biliary system, hepatic stroma, and hepatic vasculature were unaffected. Hepatocellular necrosis (Fig. 1) ranged in severity from small solitary foci of hyalinization to multifocal or diffusely distributed regions of coagulation necrosis. All four cases of hepatocellular necrosis observed among the untreated and sham-fed controls consisted of small foci of hyalinization. The remaining cases of necrosis seen in treated fish were either multifocal or diffuse coagulation necrosis. Necrosis was first observed by the end of the first week. The following is the frequency distribution of hepatocellular necrosis by lesion severity (mild, moderate, and severe) and treatment status (untreated and sham-fed controls versus cadmium and/or PCB treated):

Severity	Combined control groups	Combined treatment groups
Mild	4	40
Moderate	0	8
Severe	0	4

Chi-square comparison of lesion severity between the control and treated fish indicated that only treated fish developed serious necrotic lesions, rejecting the hypothesis of homogeneous distribution of frequencies ($P < 0.05$). By the end of the second week, foci of hepatocellular regeneration began appearing throughout the necrotic regions, preceding extensive infiltration of the hepatic parenchyma by inflammatory or phagocytic cells. Maturing regenerative hepatocytes most frequently assumed a

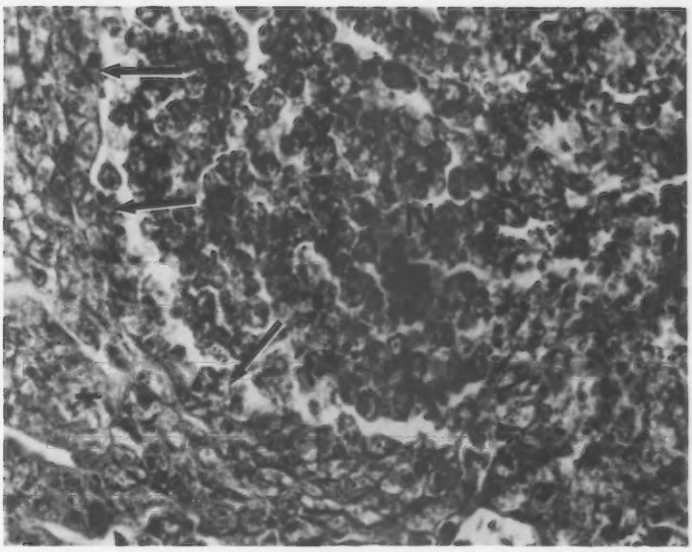


FIG. 1. Hepatocellular coagulation necrosis. A central focus of pyknosis and cellular debris is evident (N), bordered by degenerating parenchyma (arrows). From a specimen exposed to 5 μg Cd/g body wt that was killed on day 7. H&E stain. Bar represents 10 μm.

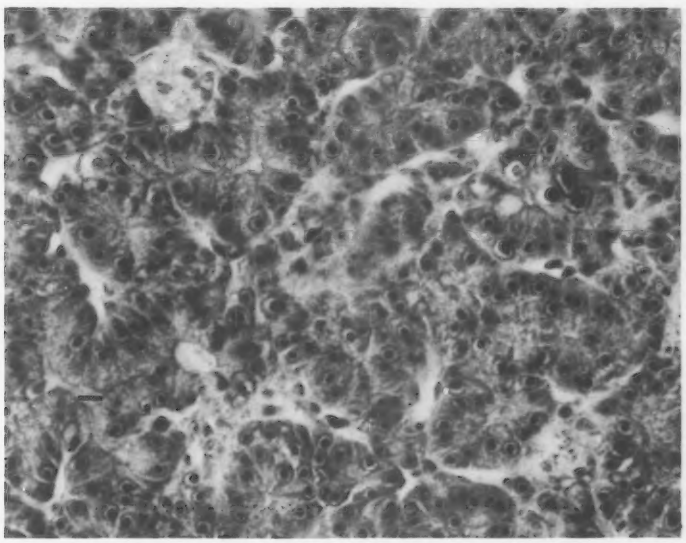


FIG. 2. Hepatocellular karyomegaly. Hepatocytes are arranged in trabecular muralia with enlarged nuclei and nucleoli. From a specimen exposed to 5 μg Cd/g body wt and 25 μg PCB/g that was killed on day 28. PTAH stain. Bar represents 10 μm.

trabecular arrangement, but occasionally took a tubular form, especially if the inflammatory reaction and fibrosis were pronounced. Degeneration of regenerated tissue was also observed. Karyomegaly, observed only among maturing parenchymal cells, was characterized by enlargement of nuclear and nucleolar diameters up to 4 times the normal diameter (Fig. 2).

In the small intestine four abnormalities were recognized: mucosal necrosis, mucous cell hyperplasia, mucosal basal cell proliferation, and mucosal pseudocyst formation. The most common early intestinal lesion was mucous cell hyperplasia, which appeared as discrete foci of mucous cell aggregates in the intestinal epithelial lining (Fig. 3). Intestinal mucosal necrosis was characterized by reduction in the height of the mucosal epithelium with pyknosis, sloughing, and occasional submucosal imflammatory infiltrates. Basal cell proliferation was identified in discrete nests of disorganized cells at the base of the mucosa. Cells within these nests displayed increased nuclear to cytoplasmic ratio and increased mitotic activity, suggesting that

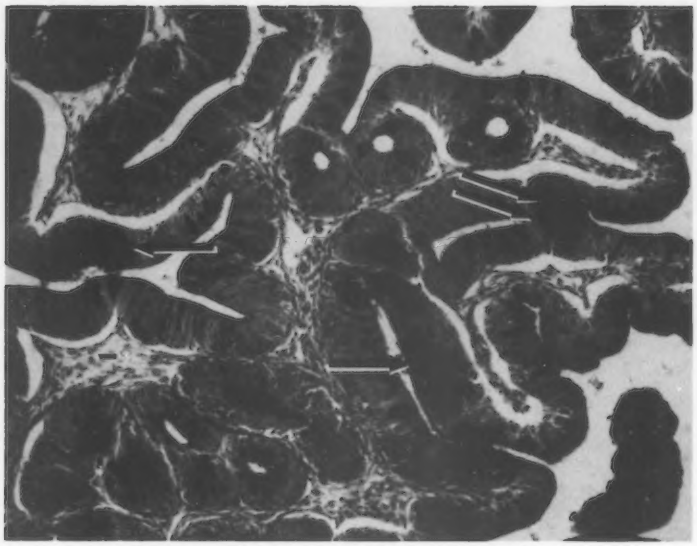


FIG. 3. Intestinal mucous cell hyperplasia (single arrows). Mucosal pseudocysts, some containing mucin, are also visible (double arrow). From a specimen exposed to 5 μg Cd/g body wt that was killed on day 21. PAS stain. Bar represents 20 μm.

they were a regenerative cell type. Mucosal pseudocysts appeared as closed pockets of mucin surrounded by proliferating mucosal cells (Fig. 3). The lumina of the pseudocysts were not contiguous with the intestinal lumen.

Several parasitic conditions were also observed in these English sole. The most common parasitic infestations and infections included *Trichodina* ciliates found usually on the gill epithelium and occasionally on the epidermis; epitheliocystis, a rickettsia-like infection of the gill epithelium; a Myxosporidian protozoan occurring frequently within the renal tubular lumina and in the hemopoietic tissues of the posterior kidney; and an *Eimeria*-like coccidian found in both the lumen and mucosa of the small intestine. All of these parasitic conditions have been previously observed in animals held at the Mukilteo facility and in English sole throughout Puget Sound (Malins et al. 1980). These infestations and infections were associated with minimal tissue damage or host response. The prevalences of the hepatic and intestinal anomalies described were not significantly altered by the presence of any of the parasites ($P > 0.05$).

Overall prevalences of all nonparasitic hepatic and intestinal lesions, except mucous cell hyperplasia, were highest among the cadmium-exposed groups (Table 2). Groups receiving cadmium only, cadmium plus PCB, and PCB only had high, intermediate, and low prevalences, respectively, of most lesions. Intestinal coccidiosis was lowest in the PCB exposure groups, intermediate in the cadmium exposure groups, and highest in the dual exposure and control groups (Table 2).

A significant effect of cadmium was observed for all lesions of concern (Table 3). Exposure to cadmium alone significantly increased the odds of lesion development for all nonparasitic conditions of the liver and intestine. For hepatocellular necrosis and intestinal mucosal necrosis, the odds increased approximately 41 and 6 times, respectively, over that of the untreated controls. On the other hand, the relative odds of intestinal coccidiosis among sole exposed only to cadmium were reduced to less than half the relative odds for the untreated sole.

Exposure to PCB alone significantly increased the odds of only one condition, hepatocellular necrosis (Table 3). The effect of PCB exposure on intestinal coccidiosis reduced the odds of infection to nearly a quarter of the odds for the untreated sole (Table 3).

Simultaneous exposure to cadmium and PCB produced evidence of an antagonistic interaction between these compounds on the odds of developing hepatic lesions (Table 3). The relative odds of hepatocellular necrosis and regeneration with simultaneous exposure were greater than for exposure to PCB alone, but much less than for exposure to cadmium alone. The antagonistic effect of PCB was most apparent for hepatocellular karyomegaly (Table 3).

An effect of the vehicle was also observed. Exposure to the agar vehicle greatly increased the odds of developing mucous cell hyperplasia compared with the untreated controls. Beyond the effect of the agar, an increase in the odds of mucous cell hyperplasia associated with cadmium exposure was detected.

Because the simultaneous and interactive effects of significant risk factors on the probability of the lesion development are often difficult to visualize, comparisons of observed prevalences and prevalences predicted by the regression models were made. Figure 4 illstrates the comparison of prevalences for the hepatic lesions and demonstrates the close correspondence of the predicted and observed prevalences for hepatocellular regeneration and karyomegaly. Corresponding plots for the intestinal conditions (not shown) suggested that appropriate regression models were employed.

The patterns of prevalence for hepatic and intestinal lesions following exposure to fuel oil were variable, and logistic regression models for abnormalities observed during this portion of the experiment revealed few histopathological alterations due to fuel oil exposure (Table 4). All lesions observed during this portion of the experiment had been diagnosed during the earlier cadmium/PCB exposure period. Although prior exposure to cadmium produced a persistent elevation in the

TABLE 2. Overall prevalences (%) of pathological conditions in liver and small intestine, including mucosal coccidia, for groups exposed to cadmium and/or PCB and unexposed to No. 2 diesel fuel (see Table 3 for results of regression analyses).

	Untreated controls	Sham-fed controls	Cadmium	PCB	Cadmium + PCB
Liver					
Hepatocellular necrosis	4.0	4.4	62.5	14.0	34.9
Hepatocellular regeneration	0	0	45.8	2.0	23.3
Hepatocellular karyomegaly	0	0	14.6	0	2.3
n	50	45	45	50	43
Small intestine					
Intestinal mucosal necrosis	8.0	2.3	25.5	2.0	14.0
Intestinal mucosal basal cell proliferation	0	0	36.2	0	27.9
Intestinal mucosal pseudocyst formation	0	0	27.7	0	18.6
Intestinal mucous cell hyperplasia	0	6.8	31.9	4.0	39.5
Intestinal mucosal coccidia	46.0	36.4	21.3	18.0	34.9
n	50	44	47	50	43

TABLE 3. Odds ratios for lesion development associated with xenobiotic exposure from logistic regression analysis. The results of Fisher's exact test for significant differences between groups having zero and nonzero lesion prevalences determined the set of experimental groups to be included in subsequent calculations of odds ratios by logistic regression methods. Probability limit for the exclusion of significant parameters is ≤0.05. (ND indicates no test performed, since lesion occurred in all exposure groups; a dash indicates no significant effect ($P > 0.05$) and therefore no odds ratio calculation; an asterisk indicates overwhelming significant effect, with a large estimated odds ratio (>100)).

Lesion description	Significant effect (p-value, Fisher's exact test)	Baseline comparison group	Cadmium exposure (odds ratio)	PCB exposure (odds ratio)	Concurrent cadmium + PCB exposure (odds ratio)	Agar vehicle exposure (odds ratio)
Hepatocellular necrosis	ND	Untreated controls	41.30	3.84	14.50	—
Hepatocellular regeneration	Xenobiotic (cadmium and/or PCB exposure, $p < 0.001$)	PCB only exposure	71.01	—	16.63	—
Hepatocellular karyomegaly	Cadmium exposure ($p < 0.001$)	Cadmium only exposure	*	—	0.12	—
Intestinal mucosal necrosis	ND	Untreated controls	5.75	—	—	—
Intestinal mucosal basal cell proliferation	Cadmium exposure ($p < 0.001$)	Cadmium only exposure	*	—	—	—
Intestinal mucosal pseudocyst formation	Cadmium exposure ($p < 0.001$)	Cadmium only exposure	*	—	—	—
Intestinal mucous cell hyperplasia	Agar exposure ($p < 0.001$)	Sham-fed controls	11.66	—	—	*
Intestinal mucosal coccidia	ND	Untreated controls	0.48	0.29	0.79	—

probability of necrosis, subsequent fuel oil exposure significantly reduced the odds of this condition (odds ratio = 0.29). Apparent differences in the prevalences of the remaining liver conditions and the intestinal lesions between animals exposed or unexposed to fuel oil were not significant. No statistical interaction between fuel oil exposure and prior cadmium or PCB exposure was observed. Among the parasitic infections and infestations, only the presence of a gill ciliate, a *Trichodina* species, showed a marked decrease due to fuel oil exposure (odds ratio = 0.03).

Serum Chemistry

Serum ASAT activity and serum concentrations of albumin,

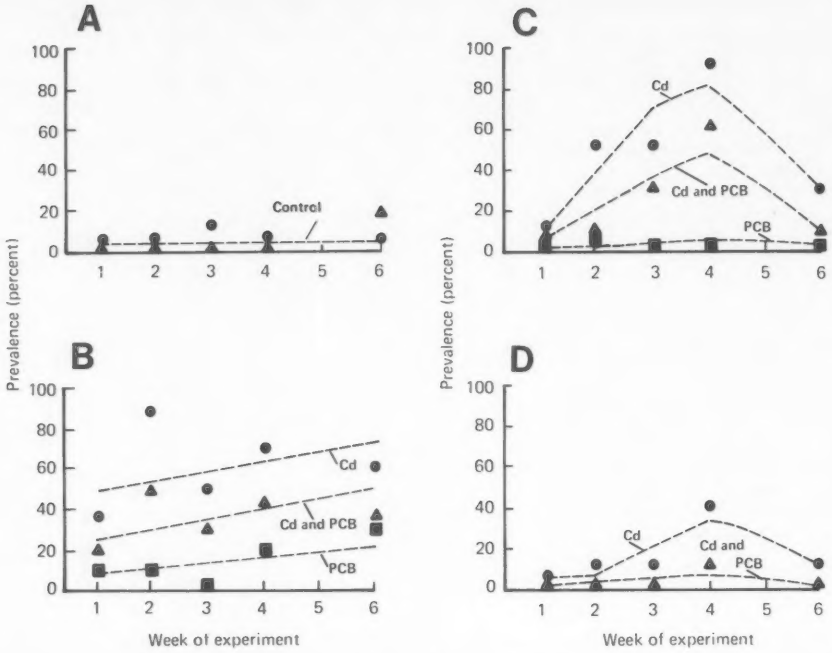


FIG. 4. Plots of observed prevalences and regression models for (A) hepatocellular necrosis in control groups and (B) hepatocellular necrosis, (C) hepatocellular regeneration, and (D) hepatocellular karyomegaly among groups exposed to xenobiotics for the first 4 wk of the experiment. Figure 4A: ▲, untreated controls; ●, sham-fed controls. Figures 4B–4D: ●, cadmium; ■, PCB; ▲, cadmium plus PCB. Regression models are indicated by broken lines.

TABLE 4. Prevalences (%) of liver lesions, small intestine lesions, and *Trichodina* infestations for groups exposed and unexposed to seawater-accommodated fraction of No. 2 diesel fuel subsequent to cadmium or PCB exposure.

	Untreated control		Sham-Fed control		Cadmium		PCB		Cadmium + PCB	
	No oil	Oil	No oil	Oil	No oil	Oil	No oil	Oil	No oil	Oil
Liver lesions										
Hepatocellular necrosis[a]	20	10	10	0	60	25	30	0	33.3	40
Hepatocellular regeneration	0	10	0	0	30	50	0	0	0	30
Hepatocellular karyomegaly	0	0	0	0	10	25	0	0	0	20
n	10	10	10	10	10	4	10	10	3	10
Small intestine lesions										
Intestinal mucosal necrosis	10	0	0	10	0	0	0	0	0	0
Intestinal mucosal basal cell proliferation	0	0	0	11.1	0	0	0	0	0	11.1
Intestinal mucosal pseudocrypt formation	0	0	0	0	0	0	0	0	33.3	11.1
Intestinal mucous cell hyperplasia	0	0	0	0	0	0	10	0	0	0
n	10	10	8	9	10	10	10	3	3	9
Trichodina infestation[b]	80	22.2	88.9	10.0	100.0	0	60.0	10.0	100.0	10.0
n	10	9	9	10	10	4	10	10	3	10

[a]Estimated odds ratio for oil-exposed fish for this condition are as follows: cadmium, 6.99; oil exposure, 0.29.
[b]Estimated odds ratio for oil-exposed fish for this condition are as follows: oil exposure, 0.03.

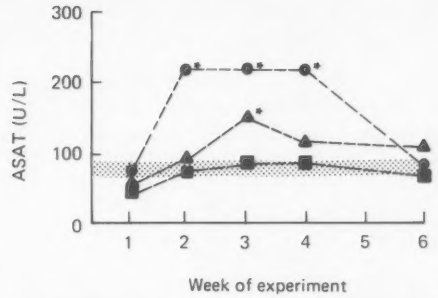


FIG. 5. Mean serum ASAT activity in English sole exposed to xenobiotics for the first 4 wk of the experiment. ●, cadmium; ■, PCB; ▲, cadmium plus PCB. The 95% confidence interval for controls throughout the experiment is represented by the shaded area. Significantly different values (ANOVA, $P \leq 0.05$) are indicated by an asterisk.

calcium, and magnesium varied depending on the type of toxicant exposure. Administration of PCB to sole did not significantly change ASAT activity relative to that of controls during the entire 6-wk period (Fig. 5); however, exposure of sole to cadmium resulted in a significant increase within 2 wk of the start of the exposure. Serum ASAT activity remained elevated through the fourth week of exposure, but returned to near control levels by the sixth week of the experiment, which was 3 wk after final exposure to cadmium. Simultaneous administration of cadmium and PCB to sole resulted in a significant increase in serum ASAT activity by the third week of exposure, but the activity returned to near control levels by the fourth exposure week.

Serum albumin concentration did not significantly change from control levels through the third week of exposure (Fig. 6). By the fourth exposure week, serum albumin concentrations were significantly lower from that of controls in all exposure groups and remained depressed through the sixth week of exposure.

Exposure to cadmium resulted in no significant change in serum calcium concentrations in English sole during the 6-wk experimental period (Fig. 7). Administration of PCB to sole resulted in a significant depression in calcium concentrations by the third week of exposure. Serum calcium returned to control levels by the fourth week. Simultaneous administration of cadmium and PCB to sole resulted in a significant decrease in serum calcium concentration during the third and fourth weeks of exposure. No samples were available during the sixth week due to extensive mortality in this group.

Serum magnesium concentration was significantly decreased in PCB-exposed sole 2 wk after the initial administration of the xenobiotic, whereas in cadmium-exposed sole, serum magnesium was significantly elevated 3 wk after the initial administration of cadmium (Fig. 8). Serum magnesium was significantly elevated in both cadmium- and PCB-exposed sole at the sixth week sampling period. Again, there were insufficient samples available at the sixth week sampling period to determine serum magnesium levels of the combined cadmium- and PCB-exposed group.

There were no significant differences in serum glucose and phosphate concentrations between control and exposed fish during the entire experimental period.

Exposure of English sole to fuel oil with or without prior exposure to cadmium or PCB resulted in variable responses

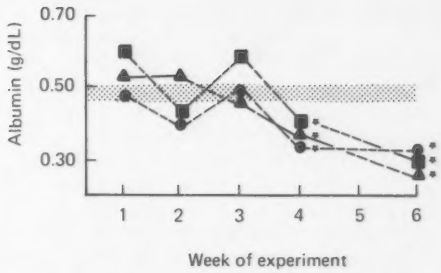


FIG. 6. Mean serum albumin concentrations in English sole exposed to xenobiotics for the first 4 wk of the experiment. Symbols are as defined in Fig. 5.

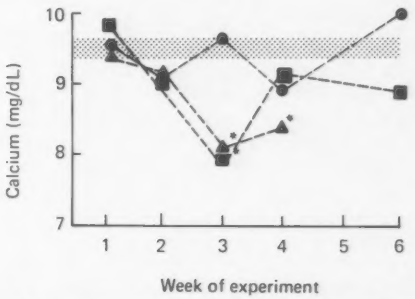


FIG. 7. Mean serum calcium concentrations in English sole exposed to xenobiotics for the first 4 wk of the experiment. Symbols are as defined in Fig. 5.

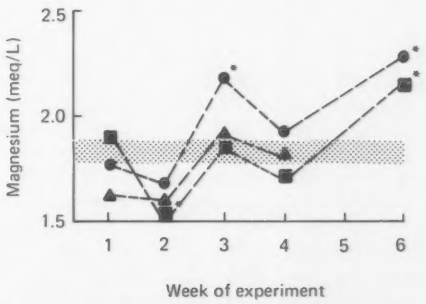


FIG. 8. Mean serum magnesium concentrations in English sole exposed to xenobiotics for the first 4 wk of the experiment. Symbols are as defined in Fig. 5.

relative to controls with respect to the serum parameters measured (data not shown).

Serum albumin concentrations were significantly decreased in all groups exposed to fuel oil except sole that had previously been exposed to PCB. Serum albumin levels in the latter group were significantly increased relative to controls.

Serum magnesium concentrations were significantly increased in all groups exposed to fuel oil except for sole that had been previously exposed simultaneously to cadmium and PCB. Although magnesium concentrations were elevated in this group the increase was not significantly higher than control levels. There were no other significant effects noted after exposure to fuel oil with respect to the other serum parameters monitored.

We used analysis of variance with respect to toxicant expo-

sure to identify possible interactive toxicant effects. Serum albumin levels of sole exposed to PCB for 4 wk and to fuel oil for an additional 2 wk were significantly elevated compared with albumin levels of sole exposed to other xenobiotics, either singly or in combination. ASAT activity of sole exposed to cadmium for 4 wk was significantly higher than levels of sole exposed to PCB or cadmium plus PCB. No other significant differences were present in any of the other serum parameters among sole exposed to any of the xenobiotics.

Comparison of the frequency distribution of sole with or without liver lesions, irrespective of the type of xenobiotic exposure, demonstrated a significant increase in serum ASAT activity and decrease in glucose concentration in sole with liver lesions compared with sole without liver lesions.

Discussion

Significant effects of independent cadmium and PCB exposure on the histology of the liver and intestinal tract and on serum chemical levels in English sole were found. Simultaneous exposure to these compounds produced statistical evidence of an antagonistic effect of PCBs on the effects of cadmium exposure. Subsequent exposure to seawater-accommodated fuel oil did not result in any further significant interactive effects on the parameters under investigation.

The lesions associated with cadmium exposure observed in other teleosts resemble those we found. Gardner and Yevich (1970) noted mucosal necrosis and sloughing in the small intestine of killifish (*Fundulus heteroclitus*) acutely exposed to high levels (50 μg/L) of waterborne cadmium chloride. Sastry and Gupta (1979) reported hepatocellular pyknosis (necrosis) as well as hyperactive mucus secretion by goblet cells found in the small intestine of the catfish, *Heteropneustes fossilis*. Because the intestinal tract and liver were anticipated to be the initial absorptive organs to encounter the metal in the present study, histopathological alterations at these sites were not unexpected. Uptake studies in other marine flatfish have indicated that cadmium concentrations in the liver and gastrointestinal tract exceeded renal concentrations under natural and experimental conditions (Wright 1976; Pentreath 1977). Significant short-term accumulation of cadmium in the gill and liver followed by a slower accumulation in the kidney of exposed salmonids was found by Roberts et al. (1979). Those studies correspond well with tissue burden data observed by our laboratory in which English sole exposed to cadmium in the diet for 3 wk were found to accumulate significant amounts of cadmium in the liver and intestinal tract but not in the posterior kidney (unpublished observations). At the dose level used in this study (5 μg/g body wt) and the duration of exposure (once weekly for 4 wk), concentrations of cadmium in the kidney may not have achieved levels or cytosolic distributions that result in detectable alterations. The effective vascular dosage of this study was probably lower than the administered dose, since absorptive efficiency of the intestinal tract for cadmium has been found to vary from 1 to 30% in mammals (Friberg et al. 1974; Valberg et al. 1977). Although there is evidence that fish possess a metallothionein-like protein involved in binding cadmium (Overnell and Coombs 1978; Reichert et al. 1979), non-thionein-bound cadmium, which may be distributed to cellular organelles and high molecular weight proteins, may also be present (Brown et al. 1982). The hepatic necrosis observed in this study may have been related to the presence of non-thionein-bound cadmium. Conversely, the lack of significant morphological changes specific to cadmium exposure in the posterior kidney may have resulted from complete binding of renal cadmium to cytosolic metallothionein.

The hepatic regenerative response found in cadmium-exposed English sole represented a natural sequelae to necrosis. The presence of this condition clearly indicated that extensive and severe damage to the liver had occurred, and its absence among the control groups suggested that the necrosis observed among control animals was of a minor and incidental nature. The morphology of the hepatic karyomegaly, found only in cadmium- and PCB-exposed sole, was very similar to a condition of nuclear pleomorphism found by Malins et al. (1984) and Myers et al. (in prep.) in English sole collected from selected sites in Puget Sound. The condition has been characterized by distinct variation in nuclear size and chromatin distribution, often with hyperbasophilia of the nuclei. It has been found to be significantly more prevalent in sole captured at sites having severely contaminated bottom sediments (Malins et al. 1980, 1984, 1985), and was considered by those investigators to be a contaminant-related lesion. The appearance of hepatic karyomegaly in only those groups exposed to cadmium and only during the latter part of the experiment suggested that repeated exposure to cadmium may have had a role in the induction of this condition. Whether the cells displaying karyomegaly were mature or regenerative hepatocytes is not known, although karyomegaly occurred only in livers displaying regenerative parenchyma.

The intestinal alterations observed in this study were suggestive of an acute injury followed by reparative proliferation, and in some cases, hyperplasia. Although nonspecific necrosis of the liver has been found in natural populations of English sole regardless of the degree of sediment contamination at the site of capture, idiopathic necrosis of the intestinal mucosa was not found to be prevalent among sole collected in field studies (Malins et al. 1980). The significant increases of mucosal necrosis and the exclusive appearance of the regenerative condition and the hyperplastic pseudocyst development in the cadmium-exposed fish strongly imply a role of cadmium in injury.

Changes in the levels of serum components have frequently been related to tissue damage or impaired organ function. Alterations in levels of ASAT activity in other vertebrate species exposed to cadmium or PCBs have been infrequently observed, and in general, changes in ASAT activity in mammals exposed to cadmium occurred only after high dosage levels. For example, Piscator and Axelsson (1970) found elevated levels of serum ASAT activity in rabbits exposed to 0.25 mg Cd/kg body wt for 5 d/wk for 24 wk, but these levels returned to normal upon discontinuation of treatment, as we found. Similarly, no significant changes in serum ASAT activity levels were found by Kasza et al. (1976) in rats acutely and subacutely exposed to Aroclor 1254 for 4 wk, as we found. Although ASAT levels in cadmium-exposed sole returned to control levels by the termination of the experiment, the prevalence of hepatocellular necrosis did not correspondingly decline. Because ASAT activity levels may reflect not only the presence of tissue damage but also the magnitude of damage, the decrease may be more closely related to the severity of liver necrosis within individual fish. Subsequent examination of severity of liver necrosis revealed a pattern of rise and decline similar to that of ASAT activity level.

Whereas the temporal trend of ASAT activity levels displayed an increase during the period of cadmium exposure

followed by a decrease with cessation of exposure, albumin concentrations did not differ from control levels until the end of the fourth week and continued to decline until the sixth week. Furthermore, depressed albumin levels were observed among all cadmium- and PCB-exposed groups. Comparisons of the temporal pattern of albumin concentrations among the three groups suggested an inverse relation to the temporal pattern of hepatocellular necrosis prevalence for the first 4 wk. Since albumin concentrations typically change as a result of longer term liver disease (Sodeman and Sodeman 1974), the later appearance of depressed albumin levels corresponds to an indication of early tissue damage and associated functional impairment.

Although serum hypocalcemia has been found in mammals (Itokawa et al. 1974) and European flounder (*Platichthys flesus*) (Larsson et al. 1981) exposed to cadmium, we did not find this effect. However, PCB exposure, both alone and in combination with cadmium, produced a decrease in serum calcium levels in English sole. Because fish having acellular bone, such as the Pleuronectidae, are reputed to possess metabolically inert mineral deposits in the skeleton (Moss 1961), serum calcium levels may be maintained primarily by regulation of calcium absorption across the intestinal mucosa. We suggest that an inhibition of calcium transport across the intestinal mucosa, similar to that observed in PCB-treated birds (Wong et al. 1974), may have been responsible, in part, for the observed decrease in serum calcium.

The anticoccidial activity of cadmium and PCB may have been a result of one or a combination of two effects: (1) the xenobiotics may have a direct toxic action against coccidia and (2) alterations in the intestinal microenvironment due to the xenobiotics inhibit or prevent infection, e.g. loss of suitable epithelia due to necrosis and sloughing or changes in pH levels. Release of infective sporozoites from *Eimeria* oocysts is known to require the presence of specific microenvironmental conditions, and infectivity can be reduced by alteration of these conditions (Cheng 1973; Chappell 1979).

The apparent antagonistic effect of PCB on the effects of cadmium toxicity we observed may be related to a potentiation of cadmium metabolism by PCB. Microsomal enzyme inducers such as phenobarbital are capable of ameliorating the effects of cadmium toxicity by a mechanism other than metallothionein induction (Yoshikawa and Ohsawa 1975), and the binding of cadmium to a low molecular weight compound for excretion into the bile can be increased by exposure to microsomal enzyme inducers (Klaassen and Kotsonis 1977). Null interactive effects of PCB with other compounds in teleosts have been reported for a variety of environmental xenobiotics including some carcinogens (Bills and Marking 1977; Hendricks et al. 1980; Hesselberg and Nicholson 1981).

Subsequent exposure to a seawater-accommodated fraction of No. 2 diesel fuel produced few histological effects and equivocal serum chemical results. In fact, subsequent exposure to diesel fuel corresponded to a decreased prevalence of hepatocellular necrosis. This observation contradicts reports of dramatic histological changes in livers of fish exposed to waterborne petroleum hydrocarbons (Solangi and Overstreet 1982), but the disparity may be attributable to variations in compositions of the hydrocarbon mixtures or differences in species susceptibility to hydrocarbon toxicity. We did not observe the vascular and hematological disorders diagnosed in the livers of coho salmon (*Oncorhynchus kisutch*) exposed to No. 2 fuel oil (Cardwell 1973) and the extensive hepatocellular lipid vacuolation observed by McCain et al. (1978) in English sole exposed to crude oil contaminated sediments; therefore, we suggest that the composition of the contaminant may be important. The reduction in hepatocellular necrosis among fuel oil exposed sole, excepting those previously exposed to cadmium and PCB simultaneously, was an unanticipated effect. However, the significant variations in serum albumin and magnesium concentrations among fuel oil exposed fish indicate that perhaps more subtle changes of biosynthetic and osmoregulatory functions occurred, and more definitive morphological alterations may have emerged with a longer duration of exposure.

Unlike Moles (1980), who reported an increased sensitivity to a glochidial ectoparasite among coho salmon exposed to waterborne crude oil, the aromatic hydrocarbon exposed fish in this study displayed decreased ectoparasitic infestation. Given the waterborne exposure route, the *Trichodina* ciliate may have a greater sensitivity to the effects of the petroleum contaminant than the vertebrate host. Waterborne exposure to toxicants, such as formalin and malachite green, has been employed commonly as a means of treating trichodiniasis in fish (Amlacher 1970).

Exposure of a marine teleost to representatives of three classes of chemical contaminants revealed differential interactive effects. Hepatic and intestinal lesions were most prevalent in animals exposed to only cadmium and relatively reduced in fish exposed simultaneously to cadmium and PCB. Similarly, serum ASAT and magnesium concentrations were highest in cadmium-exposed fish and lower in fish exposed to cadmium plus PCB. Subsequent exposure to fuel oil did not result in significant interactive effects on tissue morphology and serum component levels.

Acknowledgements

We express our appreciation to P. K. Emery for technical assistance, H. O. Hodgins and J. E. Stein for critical readings of the manuscript, and J. T. Landahl for valuable advice. Support for this project was derived from the U.S. Environmental Protection Agency (Interagency Agreement No. EPA79-D-X0514) and the Ocean Assessment Division of the National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce.

References

AMLACHER, E. 1970. Textbook of fish diseases. D. A. Conroy and R. L. Herman [transl.] TFH Publications, Jersey City, NJ, 302 p.

ARMED FORCES INSTITUTE OF PATHOLOGY. 1968. Manual of histologic staining methods of the Armed Forced Institute of Pathology. 3rd ed. L. G. Luna [ed.] McGraw-Hill Book Co., New York, NY. 258 p.

BILLS, T. D., AND L. L. MARKING. 1977. Effects of residues of the polychlorinated biphenyl Aroclor 1254 on the sensitivity of rainbow trout to selected environmental contaminants. Prog. Fish-Cult. 39(3): 150.

BRESLOW, N. E., AND N. E. DAY. 1980. Statistical methods in cancer research. Volume 1: The analysis of case-control studies. International Agency for Research on Cancer, Lyon. 338 p.

BROWN, D. A., J. D. JENKINS, E. M. PERKINS, R. W. GOSSETT, AND G. P. HERSHELMAN. 1982. Detoxification of metals and organic compounds in white croaker, p. 157–172. *In* W. Bascom [ed.] Southern California Coastal Water Resources Project Biennial Report 1981–82, 646 West Pacific Coast Highway, Long Beach, CA.

CARDWELL, R. D. 1973. Acute toxicity of no. 2 diesel oil to selected species of marine invertebrates, marine sculpins, and juvenile salmon. Doctoral dissertation, University of Washington, Seattle, WA.

CASILLAS, E., M. MYERS, AND W. E. AMES. 1983. Relationship of serum chemistry values to liver and kidney histopathology in English sole (*Parophrys vetulus*) after acute exposure to carbon tetrachloride. Aquat. Toxicol. 3: 61–78.

CHAPMAN, G. A., AND D. G. STEVENS. 1978. Acutely lethal levels of cadmium, copper, and zinc to adult male coho salmon and steelhead. Trans. Am. Fish. Soc. 107(6): 837–840.

CHAPPELL, L. H. 1979. Physiology of parasites. John Wiley & Sons, New York, NY. 229 p.

CHENG, T. C. 1973. General parasitology. Academic Press, New York, NY. 965 p.

FINGERMAN, S. W. 1980. Differences in the effects of fuel oil, an oil dispersant and three polychlorinated biphenyls on fin regeneration in the Gulf Coast killifish *Fundulus grandis*. Bull. Environ. Contam. Toxicol. 25: 234–240.

FREEMAN, H. C., G. SANGALANG, AND B. FLEMING. 1982. The sublethal effects of a polychlorinated biphenyl, Aroclor 1254, diet on the Atlantic cod, *Gadus morhua*. Sci. Total Environ. 24(1): 1–12.

FRIBERG, L., M. PISCATOR, G. F. NORDBERG, AND T. KJELLSTROM. 1974. Cadmium in the environment. 2nd ed. CRC Press, Cleveland, OH.

GARDNER, G. R., AND P. P. YEVICH. 1970. Histological and hematological responses of an estuarine teleost to cadmium. J. Fish. Res. Board Can. 27: 2185–2196.

GRAY, P. 1954. The microtomist's formulary and guide. Blakiston Co., Inc., New York, NY.

HAWKES, J. W. 1980. The effects of xenobiotics on fish tissues: morphological studies. Fed. Proc. 39: 3230–3236.

HAWKINS, W. E., L. G. TATE, AND T. G. SARPHIE. 1980. Acute effect of cadmium on the spot *Leiostomus xanthurus* (Teleostei): tissue distribution and renal ultrastructure. J. Toxicol. Environ. Health 6(2): 283–295.

HENDRICKS, J. D., T. P. PUTNAM, AND R. O. SINNHUBER. 1980. Null effect of dietary Aroclor 1254 on hepatocellular carcinoma incidence in rainbow trout (*Salmo gairdneri*) exposed to aflatoxin B1 as embryos. J. Environ. Pathol. Toxicol. 4: 9–16.

HESSELBERG, R. J., AND L. W. NICHOLSON. 1981. Influence of PCB's in water on uptake and elimination of DDT and DDE by lake trout. J. Environ. Qual. 10(3): 315–318.

ITOKAWA, Y., T. ABE, R. TEBEI, AND S. TANAKA. 1974. Renal and skeletal lesions in experimental cadmium poisoning. Arch. Environ. Health 28: 149–154.

KASZA, L., M. A. WEINBERGER, C. CARTER, D. E. HINTON, B. F. TRUMP, AND E. A. BROUWER. 1976. Acute, subacute, and residual effects of polychlorinated biphenyl (PCB) in rats. II. Pathology and electron microscopy of liver and serum enzyme study. J. Toxicol. Environ. Health 1: 689–703.

KICENIUK, J. W., G. L. FLETCHER, AND R. MISRA. 1980. Physiological and morphological changes in a cold torpid marine fish upon acute exposure to petroleum. Bull. Environ. Contam. Toxicol. 24: 313–319.

KLAASSEN, C. D., AND F. N. KOTSONIS. 1977. Biliary excretion of cadmium in the rat, rabbit, and dog. Toxicol. Appl. Pharmacol. 41: 101–112.

LARSSON, A., B.-E. BENGTSSON, AND C. HAUX. 1981. Disturbed ion balance in flounder, *Platichthys flesus*, exposed to sublethal levels of cadmium. Aquat. Toxicol. 1(1): 19–35.

LEATHERLAND, J. F., AND R. A. SONSTEGARD. 1978. Lowering of serum thyroxine and triiodothryonine levels in yearling coho salmon, *Oncorhynchus kisutch*, by dietary Mirex and PCB's. J. Fish. Res. Board Can. 35: 1285–1289.

MALINS, D. C., M. M. KRAHN, D. W. BROWN, L. D. RHODES, M. S. MYERS, B. B. MCCAIN, AND S-L. CHAN. 1985. Toxic chemicals in marine sediment and biota from Mukilteo, Washington: relationships with hepatic neoplasmas and other hepatic lesions in English sole (*Parophrys vetulus*). J. Natl. Cancer Inst. 74: 487–494.

MALINS, D. C., B. B. MCCAIN, D. W. BROWN, S-L. CHAN, M. S. MYERS, J. T. LANDAHL, P. G. PROHASKA, A. J. FRIEDMAN, L. D. RHODES, D. G. BURROWS, W. D. GRONLUND, AND H. O. HODGINS. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ. Sci. Technol. 18(9): 705–713.

MALINS, D. C., B. B. MCCAIN, D. W. BROWN, A. K. SPARKS, AND H. O. HODGINS. 1980. Chemical contaminants and biological abnormalities in central and southern Puget Sound. NOAA Technical Memorandum OMPA-2, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA. 22161. 295 p.

MAYNARD, D. J., AND D. D. WEBER. 1981. Avoidance reactions of juvenile coho salmon (*Oncorhynchus kisutch*) to monocyclic aromatics. Can. J. Fish. Aquat. Sci. 38: 772–778.

MCCAIN, B. B., H. O. HODGINS, W. D. GRONLUND, J. W. HAWKES, D. W. BROWN, M. S. MYERS, AND J. H. VANDERMEULEN. 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole (*Parophrys vetulus*), and pathological consequences. J. Fish. Res. Board Can. 35: 657–664.

MOLES, A. 1980. Sensitivity of parasitized coho salmon fry to crude oil, toluene, and naphthalene. Trans. Am. Fish. Soc. 109(3): 293–297.

MOSS, M. L. 1961. Studies of the acellular bone of teleost fish. I. Morphological and systematic variations. Acta Anat. 46: 343–362.

NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, AND D. H. BENT. 1975. Statistical package for the social sciences. McGraw-Hill Book Co., New York, NY. 675 p.

OVERNELL, J., AND T. L. COOMBS. 1979. Purification and properties of plaice metallothionein, a cadmium-binding protein from the liver of the plaice (*Pleuronectes platessa*). Biochem. J. 183: 277–283.

PENTREATH, R. J. 1977. The accumulation of cadmium by the plaice, *Pleuronectes platessa* L. and the thornback ray, *Raja clavata* L. J. Exp. Mar. Biol. Ecol. 30: 223–232.

PISCATOR, M., AND B. AXELSSON. 1970. Serum proteins and kidney function after exposure to cadmium. Arch. Environ. Health 21: 604–608.

PREECE, A. 1972. A manual for histologic technicians. 3rd ed. Little, Brown and Co., Boston, MA. 428 p.

REICHERT, W. L., D. A. FEDERIGHI, AND D. C. MALINS. 1979. Uptake and metabolism of lead and cadmium in coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. 636: 229–234.

ROBERTS, K. S., A. CRYER, J. KAY, J. F. DE L. G. SOLBE, J. R. WHARFE, AND W. R. SIMPSON. 1979. The effects of exposure to sublethal concentrations of cadmium on enzyme activities and accumulation of the metal in tissues and organs of rainbow and brown trout (*Salmo gairdneri*, Richardson and *Salmo trutta fario* L.). Comp. Biochem. Physiol. 62C: 135–140.

ROYAL STATISTICAL SOCIETY. 1978. The GLIM system, release 3. Numerical Algorithms Group, 7 Banbury Road, Oxford, OX2 6NN, England. 185 p.

RYAN, T. A., B. L. JOINER, AND B. F. RYAN. 1981. Minitab reference manual. Duxbury Press, Boston, MA. 154 p.

SASTRY, K. V., AND P. K. GUPTA. 1979. The effect of cadmium on the digestive system of the teleost fish, *Heteropneustes fossilis*. Environ. Res. 19: 221–230.

SINGH, S. M., AND P. M. SIVALINGAM. 1982. *In vitro* study on the interactive effects of heavy metals on catalase activity of *Sarotherodon mossambicus* (Peters). J. Fish Biol. 20: 683–688.

SODEMAN, W. A., AND W. A. SODEMAN. 1974. Pathologic physiology: mechanisms of disease. W. B. Saunders Co., Philadelphia, PA. 1013 p.

SOLANGI, M. A., AND R. M. OVERSTREET. 1982. Histopathological changes in two estuarine fishes, *Menidia beryllina* (Cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water-soluble fractions. J. Fish Dis. 5: 13–35.

VALBERG, L. S., J. HAIST, AND M. G. CHERIAN. 1977. Cadmium-induced enteropathy: comparative toxicity of cadmium chloride and cadmium-thionein. J. Toxicol. Environ. Health 2: 963–975.

VOYER, R. A., AND J. F. HELTSHE. 1984. Factor interactions and aquatic toxicity testing. Water Res. 18(4): 441–447.

WONG, R. G., H. G. NOWICKI, AND A. W. NORMAN. 1974. The effects of polychlorinated biphenyls on calciferol (vitamin D) mediated calcium metabolism. Pestic. Biochem. Physiol. 4(2): 170–177.

WRIGHT, D. A. 1976. Heavy metals in animals from the north east coast. Mar. Pollut. Bull. 7: 36–38.

YOSHIKAWA, H., AND M. OHSAWA. 1975. Protective effect of phenobarbital on cadmium toxicity in mice. Toxicol. Appl. Pharmacol. 34: 517–518.

Morphological Abnormalities in Chironomidae (Diptera) Larvae as Measures of Toxic Stress in Freshwater Ecosystems: Indexing Antennal Deformities in *Chironomus* Meigen

W. F. Warwick

National Water Research Institute, Western and Northern Region, 501 University Crescent, Winnipeg, Man. R3T 2N6

Warwick, W. F. 1985. Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in *Chironomus* Meigen. Can. J. Fish. Aquat. Sci. 42: 1881–1914.

An indexing scheme for classifying antennal deformations in the genus *Chironomus* is presented to illustrate the potential for using deformities in chironomid larvae as a biological screening technique for the detection and assessment of contaminants in aquatic ecosystems. The scheme utilizes six basic steps to determine an index of morphological response (IMR) for each individual larvae; these steps evaluate (1) the loss of genuine segments and/or the presence of questionable segments, (2) the reduction in length of the antenna, (3) the displacement or loss of the ring organ, (4) the fusion of the apex of the basal segment, (5) the displacement of accessory organs, and (6) the presence of structures of unknown homology. The values for IMR are summed and divided by the total number of larvae examined to calculate an index of severity of antennal deformation (ISAD) for the sample population. Comparison of the incidence (percent) and severity of antennal deformations (ISAD) in *Chironomus* populations from Tobin Lake (contaminated) and Last Mountain Lake (comparatively uncontaminated) ecosystems in south-central Saskatchewan show that both indices are higher in Tobin Lake. Improved mounting procedures are presented in which specimens are fully flattened to improve visibility of fine detail and enhance analytical consistency. Some results from preliminary dose–response experiments using 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) are presented. These results suggest that larval antennae are very sensitive to low-level, chronic exposure to toxic chemicals. New receptor organs are also described which make the morphology of the antennae of Chironomini and Tanytarsini directly comparable.

On présente une méthode de catalogage pour classifier les déformations d'antennes chez le genre *Chironomus*, afin de montrer qu'il est possible d'utiliser les difformités observées chez les larves de chironomidés comme technique de dépistage biologique permettant de déceler et d'évaluer les contaminants présents dans les écosystèmes aquatiques. La méthode fait appel à six étapes principales en vue d'établir un indice de réaction morphologique (IRM) pour chaque larve; dans le cadre de ces étapes, on évalue (1) la perte de segments naturels ou la présence de segments suspects, (2) la diminution de la longueur de l'antenne, (3) le déplacement ou la perte de l'organe annulaire, (4) la fusion de l'extrémité du segment basal, (5) le déplacement des organes accessoires et (6) la présence de structures ayant une homologie inconnue. Les valeurs de l'IRM sont additionnées puis divisées par le nombre total de larves examinées afin d'obtenir un indice de la gravité de la déformation des antennes (IGDA) pour la population échantillon. La comparaison de la fréquence (pourcentage) et de la gravité des déformations d'antennes (IGDA) chez les populations de *Chironomus* des écosystèmes du lac Tobin (contaminé) et du lac Last Mountain (qui, par comparaison, n'est pas contaminé) dans le centre-sud de la Saskatchewan montre que les deux indices sont plus élevés dans le lac Tobin. On présente des techniques améliorées de montage, où les spécimens sont complètement aplatis pour permettre de mieux voir les fins détails et de rendre l'analyse plus cohérente. On présente quelques résultats d'expériences préliminaires de dosage du dichloro-1,1 di-*p*-chlorophényl-2,2 éthylène (DDE). Ces résultats donnent à penser que les antennes des larves sont très sensibles à de faibles concentrations de substances chimiques toxiques et à une exposition prolongée à ces produits. On décrit également de nouveaux organes récepteurs qui permettent de comparer directement la morphologie des antennes de chironomini et de tanytarsini.

Received February 11, 1985
Accepted August 6, 1985
(J8114)

Reçu le 11 février 1985
Accepté le 6 août 1985

Morphological deformities in chironomid larvae have been attributed to industrial and agricultural contamination, but to date, no systematic approach for reporting these phenomena in the literature has been devised. The present paper is the first in a series designed to establish a conceptual framework within which the broad range of deformities possible in chironomid larvae can be documented and compared. The formulation of such a framework is a prerequisite to developing a biological screening technique for detecting and assessing the significance of contaminants in aquatic environments.

Morphological deformities undoubtedly occur in populations

of chironomid larvae living in uncontaminated environments. This seems reasonable because even robust, healthy populations of any fauna are likely to include a certain proportion of physiologically weaker individuals which, for various reasons, may be more prone or genetically predisposed to malformation. However, deformities in unstressed populations probably occur less frequently and are less severe than those living under more stressed conditions.

Hamilton and Saether (1971) formulated the hypothesis that deformities in chironomid larvae were caused by environmental contaminants because deformed larvae generally were found in areas receiving industrial and/or agricultural contaminants, but not in areas polluted by domestic sewage. The Hamilton/Saether hypothesis has largely been substantiated by other authors (Brinkhurst et al. 1968; Cook and Veal 1969; Saether 1970; Hamilton and Saether 1971; Saether and McLean 1972; Hare and Carter 1976; Warwick 1980a, 1980b, 1980c; Koehn and Frank 1980; Tennessen and Gottfried 1983; Weiderholm 1984; Cushman 1984) who have found morphologically deformed chironomid larvae in lakes and streams receiving various industrial and/or agricultural contaminants; however, the direct linkage between deformities and contaminants still remains to be confirmed.

Hamilton and Saether (1971) suggested that investigation of morphological abnormalities in individual organisms might prove particularly useful in determining the biological effects of contaminants in aquatic ecosystems. Although comparatively little is known about how individual organisms react to contaminants, Petersen and Petersen (1983) suggested that changes at the organism level might be more useful than changes at the community level for purposes of environmental monitoring because individual responses occur before community responses and thus could provide an earlier warning of contaminant stress.

The primary objective of this paper is to begin to develop a framework within which morphological deformities in chironomid larvae can be used to detect and assess the nature, extent, and significance of contaminants in freshwater ecosystems. The potential for abnormal variation in an animal as complex as a chironomid larva is immense, and the number of possible expressions of response to chemical stressors is correspondingly high. Deformities described in the literature range from mildly abnormal mouthparts to grotesque thickening and fusing of all body structures (Hamilton and Saether 1971). Deformities in the larval mouthparts may range from mild asymmetry in the numbers of lateral teeth on either side of the tripartite median tooth, to overlapping of the outer lateral teeth, overlapping of the first lateral with the median tooth, or more extensive disorganization of the median or lateral areas (Warwick 1980c). Deformed mandibles often have apical teeth added or deleted, fused, or stunted. The antennal deformities described in this paper range from slight shortening of the basal segment to more severe reduction in the length of one or all segments. In severely stunted antennae, the ring organ is often lacking and the wall structure greatly thickened. The subapical teeth of the premandibles may be shorter than the main tooth, but occasionally they are much reduced in length, absent, or accompanied by additional teeth. Epipharyngeal pecten are often fused, separated, and disorganized or stunted and cramped. To date, the usefulness of these deformities as a tool for monitoring environmental contamination has been limited by the paucity of comparable data, the variation in preparation techniques, and a lack of standardized terminology and analysis.

A second objective is to standardize methods of specimen preparation so that the results of researchers working in different problem areas can be accurately compared. Because the traditional methods of preparation and mounting present the specimen in its natural configuration, the thickness of the slide generally precludes the use of high-power oil immersion lenses for detailed analysis. The methods proposed here involve flattening specimens fully so that full advantage can be taken of the level of detail obtainable at higher microscope magnifications.

This paper is confined to investigating deformations in the antennae of the genus *Chironomus*. The antennae of *Chironomus* were chosen as the starting point for the development of a biological screening tool for several reasons: (1) intuitively, receptor organs such as antennae would be very sensitive to low level contamination; (2) the impact of contamination is more readily quantifiable in the antennae than in other features of the head because of the set number of segments normally present; (3) the genus *Chironomus* is one of the most common and well-documented chironomid genera; (4) recognition of abnormal specimens within a particular genus requires a high degree of familiarity with the genus to be able to distinguish between normal and abnormal variation; and (5) deformities are known to occur in other genera but they appear to be more common in detritivores such as *Chironomus* which graze directly on contaminant-laden organic detritus (Hare and Carter 1976).

Materials and Methods

The larva I used are from culture experiments and from sites in Tobin Lake and Last Mountain Lake, south-central Saskatchewan (Fig. 1). Tobin Lake is a reservoir on the mainstem of the Saskatchewan River formed in 1962 by impoundment of the Saskatchewan River behind the Squaw Rapids hydroelectric dam. It receives a variety of chemical contaminants and toxic residues, both agricultural and industrial, from throughout the greater prairie region. As many as 5000 chemical compounds may make up the "chemical soup" to which the chironomid larvae of Tobin Lake are exposed (D. A. Birkholz, Environmental Protection Service, Edmonton, Alta. T5K 2J5, pers. comm.); of these, only 52 have so far been identified (Samoiloff et al. 1983).

Bulk samples (composites of 7–8 Ekman grabs) were collected in duplicate from Site V (53°37.9′N, 103°36.1′W) and Site VI (53°37.7′N, 103°31.1′W) in Tobin Lake on 16 October 1979 by Environmental Protection Service personnel from Regina, Saskatchewan. The two sites were located approximately 5.6 km apart in the old submerged Saskatchewan River channel in 14.3 and 23.5 m water depth, respectively. The samples were returned to this laboratory immediately after collection and sieved through 200-μm-mesh screens. Live benthic organisms, including chironomid larvae, were sorted in white enamel sorting trays from attendant debris in small amounts at a time. The debris was then searched under a stereomicroscope to check the efficiency of the sorting procedure. Individual taxa were identified, enumerated, and preserved in 70% ethanol for further analysis.

Last Mountain Lake lies in an ancient spillway cut by the drainage of glacial Lake Elstow into the Qu'Appelle River valley. It is a comparatively uncontaminated system, but during spring runoff, flood waters from the Qu'Appelle River are shunted back into the lake to alleviate downstream flooding. Industrial contaminants introduced into the Qu'Appelle River from upstream sources could therefore enter the lower reaches of Last Mountain Lake. The extent of contamination is difficult

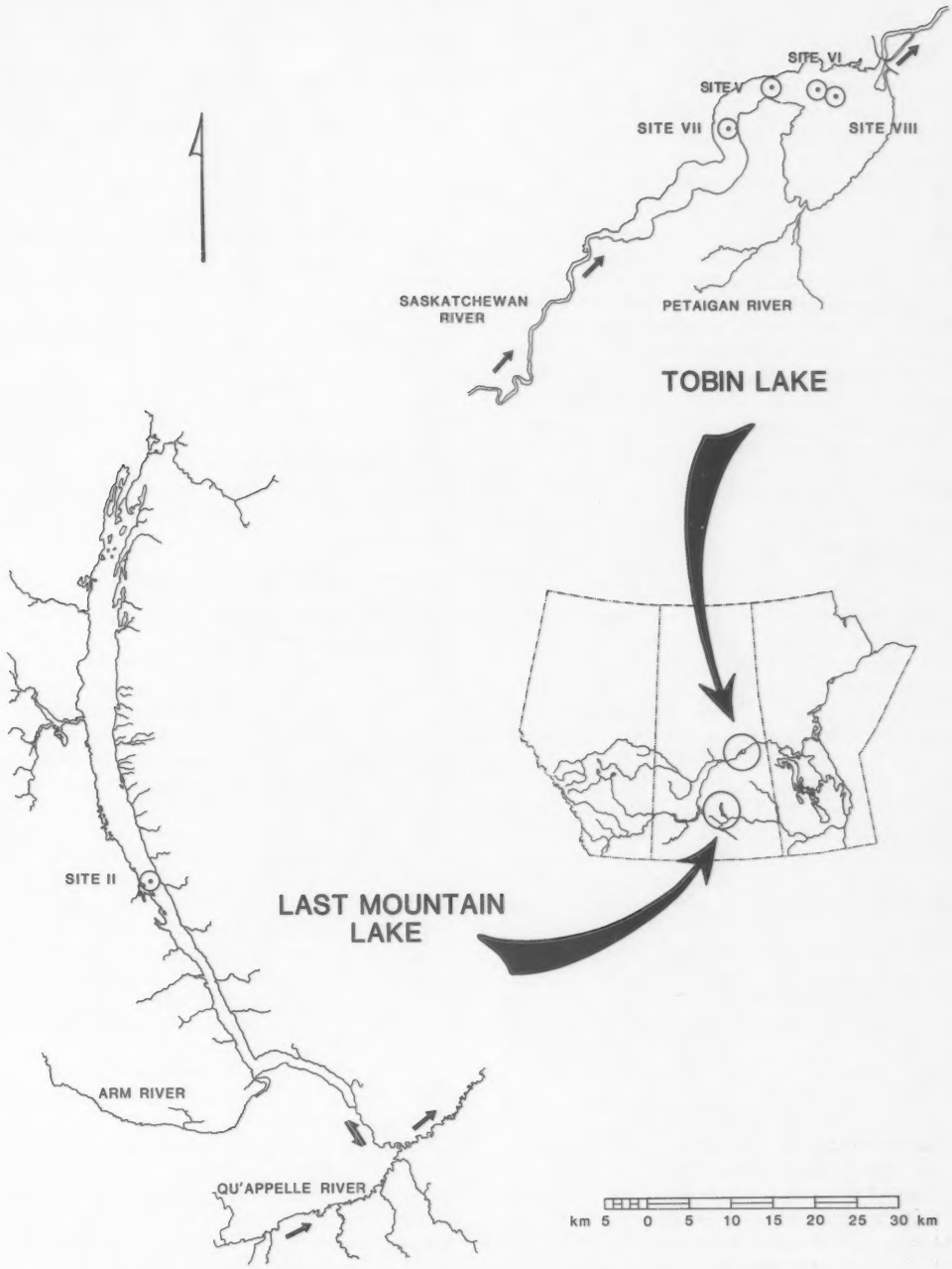


FIG. 1. Location of field sites in Tobin Lake and Last Mountain Lake, south-central Saskatchewan (adapted from Birkholz et al. 1980).

to estimate, but circulation patterns probably confine any contaminants from this source to the lower southern reaches of the lake. The samples examined for this study were collected towards the northern end of Last Mountain Lake where exposure to man-made chemicals is probably minimal. No chemical data is available at this time, but some contamination from agricultural sources and atmospheric input might be expected in this part of the lake because it lies in a highly developed agricultural area.

Bulk samples were collected in duplicate from Site II (50°59.6′N, 105°12′W) in Last Mountain Lake on 8 May 1980 by personnel from the National Water Research Institute, Winnipeg, Manitoba. Water depth was 21 m. Samples were collected using a 232-cm^2 tall Ekman grab, sieved in the field

through 200-μm-mesh nets of Nitex nylon, preserved in 70% ethanol, and returned to the laboratory. Preserved benthic organisms were stained with Rose Bengal and sorted from attendant debris in the manner described above.

Preserved larvae were mounted in Canada balsam according to the methods of Warwick (1980c) and Warwick and Casey (1982). The larvae were cleared by digesting in either warm or cold 8% KOH. The time required for clearing in warm KOH is largely dependent on the size of the individual specimen; clearing with cold KOH requires a longer period of time, but can be used effectively to control the cycling of specimens through the preparation process. The gut and body contents were removed by piercing the body wall, removing the gut tract, and flushing out dissolved body fluids. Cleared larvae were then placed in successive baths of glacial acetic acid, anhydrous ethanol, ethanol layered over cedarwood oil, and cedarwood oil for approximately 10–15 min in each bath. The head and bodies of medium to large larvae generally were mounted separately; smaller larvae were mounted intact. Dissection of the head capsule normally was carried out on the glass slide in Canada balsam rather than at the cedarwood oil stage to prevent loss of parts during subsequent manipulations. The head was placed ventral side up and the mandibles snapped back to prevent them from obscuring the antennae. The coverslip was put in place and the head capsule flattened by exerting increasing pressure to the coverslip with a pair of forceps. A rotary motion may be applied to the coverslip during this process to spread or shift mouthparts that threaten to obscure other diagnostic features.

The methods described here differ only marginally from traditional methods of Schlee (1966) and Saether (1969). Flattening and dispersal of the structures of the head capsule and larval body permit the examination of fine morphological detail and the measurement of individual structures using high-power oil immersion objective lenses. Too often, the thickness of the mount precludes detailed examination of larvae mounted fully flexed in the traditional manner. Some traditional measurements are precluded by flattening, but measurements such as head capsule width can be replaced by other measurements such as mentum width.

Larvae of *Chironomus tentans* Fab. experimentally exposed to different concentrations of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) by Hamilton and Saether (1971) were reexamined after the specimens were removed from their original Canada balsam mountings by softening in xylene vapor and remounting according to the techniques described. Larvae from master culture collections sampled at the same time as experimental controls were similarly remounted.

Most of the chironomid larvae collected from the deeper-water sites in Tobin Lake belonged (Table 1) to the genus *Chironomus*: only 2 specimens of *Procladius* spp. were found among the 1178 larvae collected. Several species of *Chironomus* undoubtedly were included in these samples. Mason and Lehmkuhl (1983) listed three species of *Chironomus* including *C. anthracinus* Zett., *C. decorus* Joh., and *C. plumosus* (L.) from a site in the reservoir located near Site VI. They also listed three other unidentified species of *Chironomus* from the Saskatchewan River upstream of Tobin Lake; these latter types, however, appear to be riverine species and probably do not figure in the lake community. Larvae of *Chironomus* spp. represented about 90% of the total fauna collected in Last Mountain Lake (Table 1); larvae of *Procladius* spp. and *Cryptochironomus* sp. made up the remaining 10% of the fauna.

TABLE 1. Summary of the types and numbers of chironomid larvae sampled in Last Mountain Lake and Tobin Lake sites, south-central Saskatchewan.

Taxon	Last Mountain Lake		Tobin Lake			
	IIa	IIb	Va	Vb	VIa	VIb
Chironomus anthracinus type	287	313	338	346	215	238
C. plumosus type	84	84	—	4	16	19
C. thummi type	45	21	—	—	—	—
C. salinarius type	1	—	—	—	—	—
Chironomus spp.	2	—	—	—	—	—
Cryptochironomus sp.	3	2	—	—	—	—
Procladius spp.	37	56	2	—	—	—
Total	459	476	340	350	231	257

For the purposes of this paper, no attempt has been made to identify larvae to species because of the problems involved in the identification of chironomid immature stages (cf. Lindeberg and Wiederholm 1979).

Structure of Normal Antennae

The antennae of chironomid larvae are complex sensory organs bearing multiple sensillae which serve as mechano- and chemoreceptors. The morphology and function of these complex organs as they are currently understood are discussed here because the proposed scheme to classify the severity of antennal deformations is based on the progressive loss of antennal segments and the degeneration of accompanying sensory structures as a measure of larval response to stress.

The structures of the five-segmented antenna of normal *Chironomus* spp. larvae is shown in Fig. 2. The terminology employed generally follows Saether (1980). Two new structures have been found, however, which apparently have not been identified previously in the literature. These are two tiny, dark-colored, reniform to conical-shaped receptors lying on the articulating membrane linking the second and third antennal segments. These receptors attach roughly at the same level as the style (St) and generally are shielded from view by the coned fairing formed by the setiform organs of Lauterborn (LO) that fringe the distal rim of the second antennal segment. They are vaguely apparent in the SEM photomicrograph presented by Sublette (1979, fig. 1), but the image is poorly defined because of the masking organs of Lauterborn.

These new structures have been identified tentatively as the peg sensilla (PS) of the Chironomini on the basis of their relationship with the organs of Lauterborn (B. Bilyj, Freshwater Institute, Winnipeg, Man. R3T 2N6, pers. comm.). The relationship makes them directly comparable with the same organs in the Tanytarsini except that the latter are mounted on stalked petioles. These new receptors appear porous in some *Chironomus* specimens, suggesting a chemoreceptor role. The peg sensilla probably serve in the general olfactory sense rather than in contact chemoreception because the overlying organs of Lauterborn shield them from direct contact with the environment. Zacharuk (1980) indicated that multiporous chemosensilla with pitted surfaces do not normally exhibit mechanosensory capabilities.

The style is a rigid, thick-walled, blade-shaped receptor

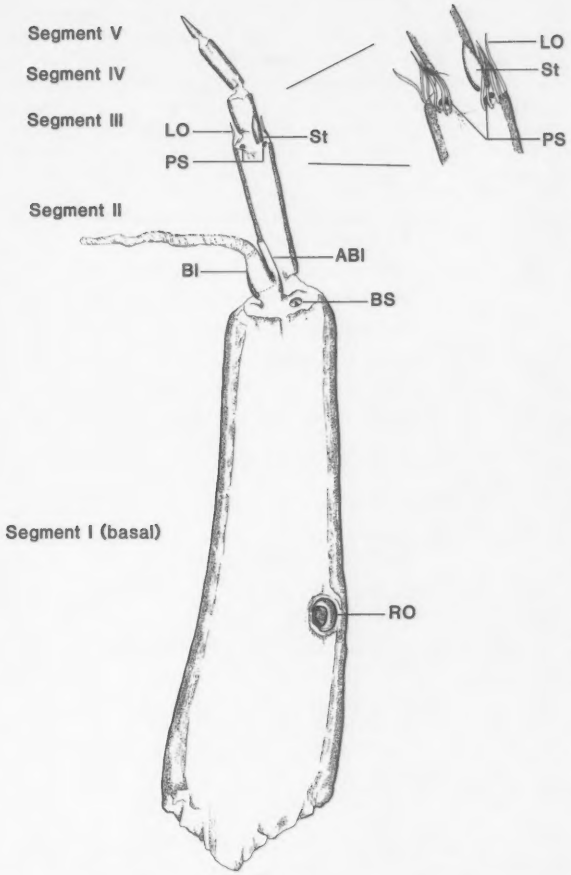


FIG. 2. Morphology of normal antennae of *Chironomus* spp. larvae. RO, ring organ; BS, basiconic sensillum; Bl, blade; ABl, accessory blade; LO, Lauterborn organs; St, style; PS, peg sensilla.

roughly equivalent in length to the third antennal segment. It is attached to the articulating membrane linking the second and third antennal segments. Saether (1980) referred to this structure as the peg sensillum, but our reinterpretation makes it more analogous with the style on the antennae of the Tanytarsini. There is no distinct socket at the point of attachment and no apparent pore opening at the tip to suggest chemosensory capability. Sublette (1979) interpreted the thick-walled style (peg sensillum; sensu Saether) as a probable proprioceptor for the remainder of the antennal segments.

The Lauterborn organs (LO: sensu Saether) are a ring of hairlike bristles that fringe the distal rim of the second antennal segment. They curve inwards to touch the wall of the third antennal segment and form a fairing over the articulating membrane linking the two antennal segments (Sublette and Sublette 1974, fig. 23). These bristles may either be chaetic or trichoid sensilla, but without an electron microscope, it is difficult to distinguish between the two types of sensilla and determine their function. Chaetic sensilla generally serve only as mechanoreceptors whereas trichoid sensilla may serve in both chemo- and mechanoreceptor roles. Sublette (1979) presumed a chemoreceptor role for the Lauterborn organs, but the morphological arrangement of the sensilla suggests a fine tactile function for control of the three distal segments of the antennae, with control of the coarser movements of the distal segments vested in the style.

The ring organ (RO) in *Chironomus* spp. is a campaniform-like sensillum comprising a dome-shaped lens lying in a circular depression located on the lower half of the basal segment (Sublette and Sublette 1974, fig. 24). Snodgrass (1935) and McIver (1975) described campaniform sensilla as proprioceptors that respond to mechanical stress in the integument. Sublette (1979), however, cautioned that the ring organ on larval antennae may not be homologous with adult campaniform sensilla because the central disc appears to be perforated.

A small cup-shaped sensillum is located near the distal end of the basal segment. This sensillum, observed only infrequently in the present material, appears under the light microscope to be equipped with a small peg such as that shown in Sublette's (1979) SEM photomicrograph of the antennae of *Maoridiamesa insularus* Brundin. Sublette (1979) interpreted the structure as a basiconic sensillum (BS), but suggested that it may be a campaniform sensillum because of its proximity to the antennal joint. If the latter interpretation is correct, the sensillum may serve as a mechanoreceptor rather than a chemoreceptor.

The antennal blade (Bl) and conjoined accessory blade (ABl) are located on the apex of the basal segment beside the remaining antennal segments. The basal portion of the blade is relatively thick-walled compared with the remaining distal portion which is of thin-walled, spiral-angled, tubular construction. Sections of the thin-walled portion frequently separate into semidetached units of coils of varying number during the mounting process. Although it is free-standing rather than looped, the finer structure of the filamentous blade of *Chironomus* spp. appears similar to that of the circumfila on the antennae of the sorghum midge *Contarinia sorghicola* Coquillett (Slifer and Sekhon 1971, fig. 25). Slifer (1970) categorized these sensilla as thin-walled chemoreceptors.

The accessory blade is a relatively thick-walled tube with a single large pore opening at the tip. Slifer (1970) stated that thick-walled receptors of this type serve as contact chemoreceptors sensitive to stronger stimuli. Zacharuk (1980) indicated that thick-walled uniporous chemoreceptors often have a mechanosensitive dendrite, but thin-walled multiporous chemoreceptors generally do not. The paired chemoreceptors on the antennae of *Chironomus* spp. possibly function in concert, with the blade serving in a slow-acting, general sensory capacity and the accessory blade serving in a more direct contact sensory capacity. The accessory blade may have secondary mechanosensory capabilities as well.

There is no evidence of pore openings at the tip of the fifth antennal segment of *Chironomus* spp. to suggest chemosensory capacity, but the fifth segment of species of *Tanytarsus* appears under light microscopy to be perforated by a subapical row of slits. Ultrastructure work by Zacharuk and Blue (1971) showed that the tip of the basiconic peg organ on the antennae of the yellow fever mosquito *Aedes aegypti* (L.) was perforated by six short slits which allow chemical molecules direct access to the dendritic units within the lumen of the peg. They suggested that the peg organ functions primarily as a contact chemoreceptor, but indicated that secondary mechanosensory capabilities were also possible. If the fifth antennal segment of *Chironomus* spp. is similarly perforated, it may possess similar capabilities.

Indexing the Severity of Antennal Deformation

Description of Deformed Antennae

The deformities I describe in this paper range from mild to bizarre. The most obvious deformities involve the loss of individual segments, the fusion of one or more adjacent segments, or the presence of segments of questionable equivalence. Individual antennae in many cases are reduced in length; these reductions can occur generally or be confined to basal, distal, or individual segments. In some cases, the ring organ may be displaced or entirely lacking. Other sensory organs such as the blade/accessory blade complex or the style may be displaced from their normal positions at the apices of the first (basal) or second segments, respectively. In more unusual cases, structures appear on deformed antennae for which no homology is known. These latter range from simple lumps and bumps to complex trident- or palmate-shaped structures. Based on experience, other unusual structures or combination of structures undoubtedly will be encountered as our data base is expanded.

Overview of Classification of Deformed Antennae

Six basic steps are involved in the assessment of the severity of antennal deformities. These are as follows:

Step 1: assign antennae to basic classification categories based on the numbers of genuine and/or questionable segments present
Step 2: assess any reduction in antennal length
Step 3: assess the status of the ring organ
Step 4: assess degree of fusion at apices of basal segment
Step 5: assess the displacement of accessory organs
Step 6: assess the presence of abnormal structures of unknown homology

The severity of individual deformities is assessed to provide a cumulative ranking of the morphological response to stress according to the arbitrary scales established below.

Step 1: Basic Classification Categories

Deformed antennae initially are assigned to basic classification categories according to the number of genuine and/or questionable segments present (Table 2). The presence of questionable segments is indicated by the question marks in the sequential format, and successive cumulative deformations are assigned numerical rankings of odd numbers starting from 1 and extending to 21. The rules for evaluating missing segments are relatively straightforward, but the rules for questionable segments are more complex because of the difficulty of interpreting nonstandard configurations. For the sake of clarity, the two subcategories will be discussed separately.

Classification of missing segments

The initial classification categories are based on the number of antennal segments present (Fig. 3). The antenna in Fig. 3b is not radically different from the normal specimen in Fig. 3a (in this case the companion antenna from the same specimen) except that the proportions[1] of the antennal segments, particularly the basal segment, are different. All five segments are present and morphologically well-formed. In Fig. 3c, 3d, and 3e, however, the number of segments is progressively reduced to two identifiable segments as the variation in morphological structure becomes more extreme. In order to be placed in category 1, the basal segment must support some identifiable morphological structure at its apex. (The term "identifiable structure" is used in the sense that the structure conforms to the general configuration described in the preceding section for normal antennae.) The antenna shown in Fig. 3f bears only an antennal blade with its heavily sclerotized base and filamentous distal portion; the accessory blade and all other segments are lacking. In contrast, the antenna in Fig. 3g is bereft of any recognizable segments, blades, or accessory blades and is placed in category 1(?). This antenna is completely "bald" and the apex of the segment completely fused over by the sclerotized wall of the segment. The ring organ is present as is a secondary structure of unknown homology. The pair of antennae in Fig. 3h are from the same larva. The antenna on the right has only three segments remaining, while that on the left has two identifiable segments and possibly a third. The ring organ, although not shown in all cases, was present on each antenna illustrated in Fig. 3.

The increasing degree of severity in antennal deformation is apparent in Fig. 4, 5, and 6, in the degree of disorganization both between and within each basic classification category. The deterioration continues to the point where there are only one or two identifiable structures located on the apex of the basal segment (Fig. 7) or identifiable structures are entirely lacking (Fig. 8). For example, the blade in Fig. 8c has no sclerotized basal portion whereas the blade in Fig. 7e does. The latter blade, therefore, is regarded as a true blade whereas the former is not.

TABLE 2. Basic classification categories for indexing the severity of antennal deformities in *Chironomus* spp. larvae based on the number of actual and/or questionable segments present.

Basic classification category (BCC)	Description	Basic index number (BIN)
5	Five segments present; noticeable reduction in length or other aberrant features present	1
5(?)	Four genuine segments plus one questionable (?) segment present apically or subapically	3
4	Four genuine segments present	5
4(?)	Three genuine segments plus one questionable (?) segment present	7
3	Three genuine segments present	9
3(?)	Two genuine segments plus one questionable (?) segment present	11
2	Two genuine segments present	13
2(?)	One genuine segment plus one questionable (?) segment present	15
1	Basal segment present; apex must support some recognizable receptor(s)	16
1(?)	Apex of basal segment present; apex supports no recognizable receptor(s)	19
0	No segments present; in practice, application impractical because of possibility of physical loss prior to, or during, mounting process	21

[1]All antennae are drawn to constant scale.

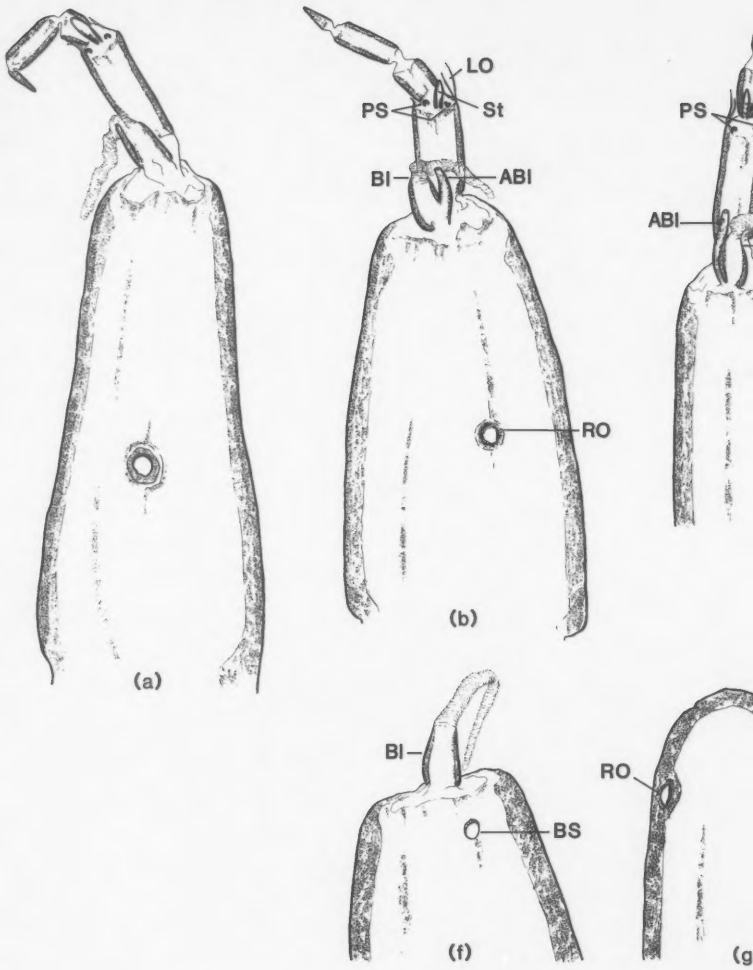
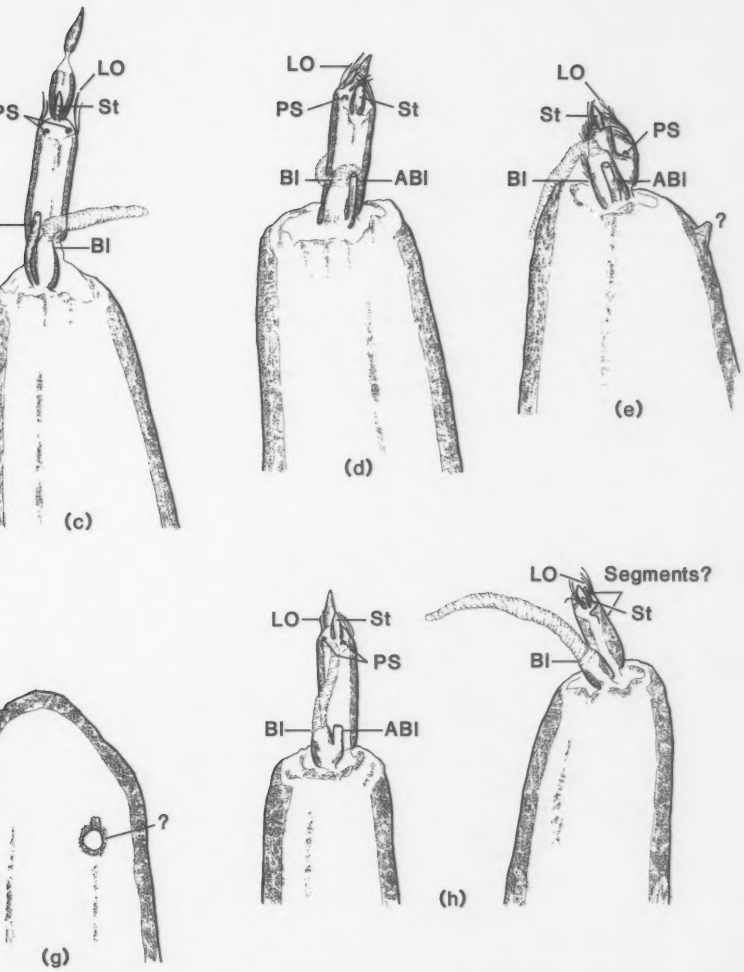
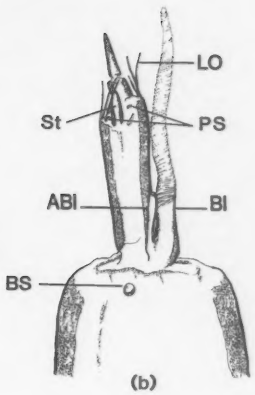


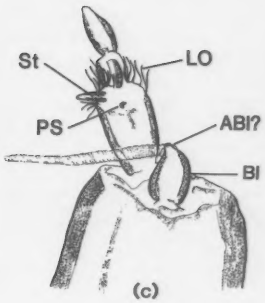
FIG. 3. Basic classification categories of deformed antennae in *Chironomus* spp. showing progressi
3, (e) category 2, (f) category 1, (g) category 1(?), and (h) category 3 and 3(?) antennae on the sam

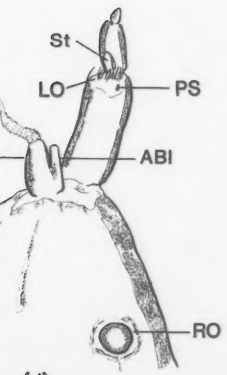


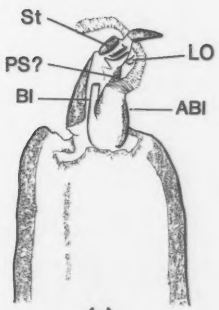
rogressive loss of antennal segments: (a) normal antenna, (b) category 5, (c) category 4, (d) category
the same larva (BIN 20).

FIG. 4. *Chironomus* spp. category 4 antennae with one



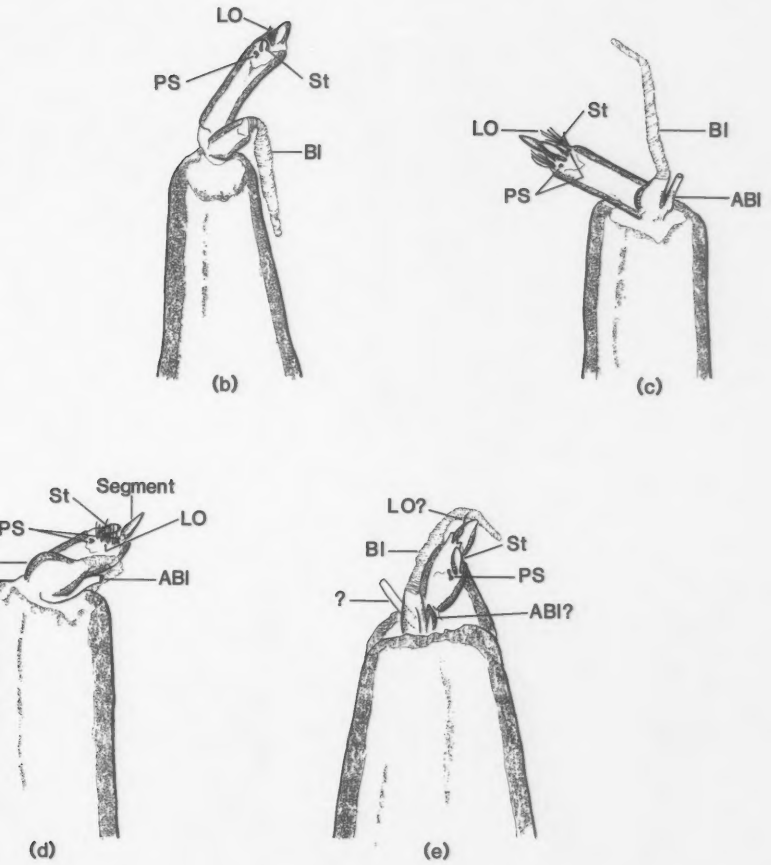






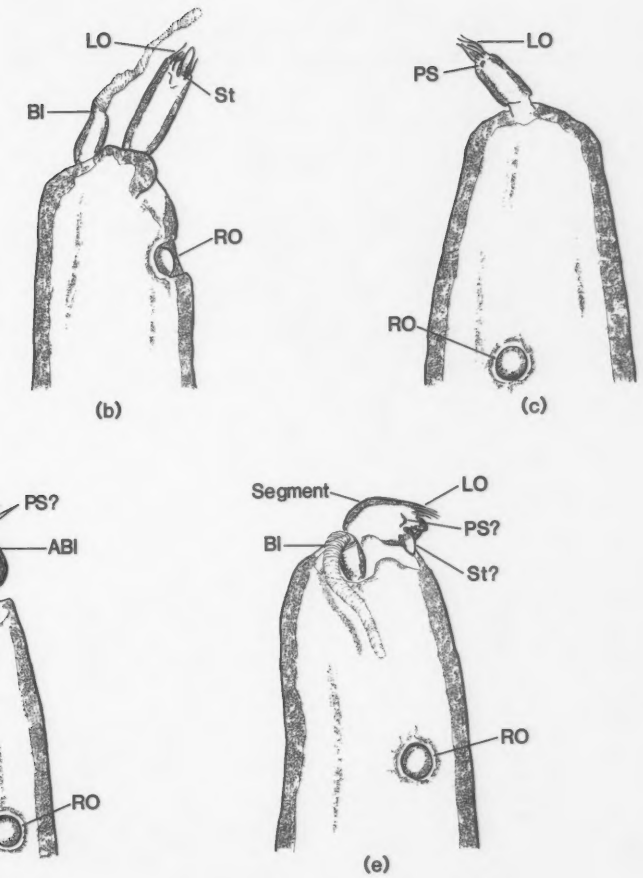
with one basal and three apical segments: (a) normal and (b–e) deformed antennae.

FIG. 5. *Chironomus* spp. category 3 antennae with one basal and two apical segments: (



gments: (a) normal and (b–e) deformed antennae. Note the organs in Fig. 5e whose exact identity is questionable.

FIG. 6. *Chironomus* spp. category 2 antennae with one basal and c



al and one apical segment: (a) normal and (b–e) deformed antennae.

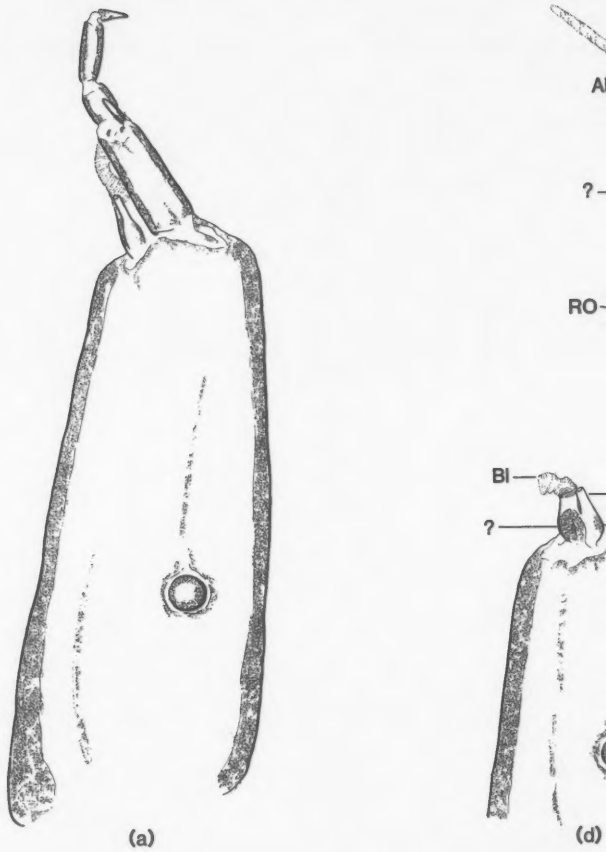
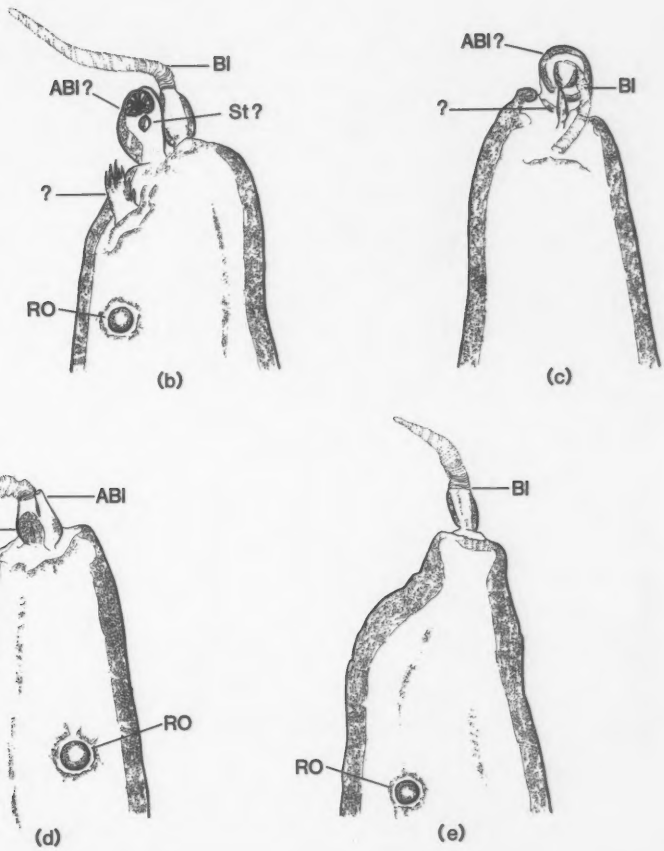


FIG. 7. *Chironomus* spp. category 1 antennae with one basal segment bearing some recognizab
blades. Note the number of organs whose identity is questionable, particularly the palmate-struct



cognizable receptor(s): (a) normal and (b–e) deformed antennae bearing (mostly) blades and accessory
ate-structure of unknown homology in Fig. 7b.

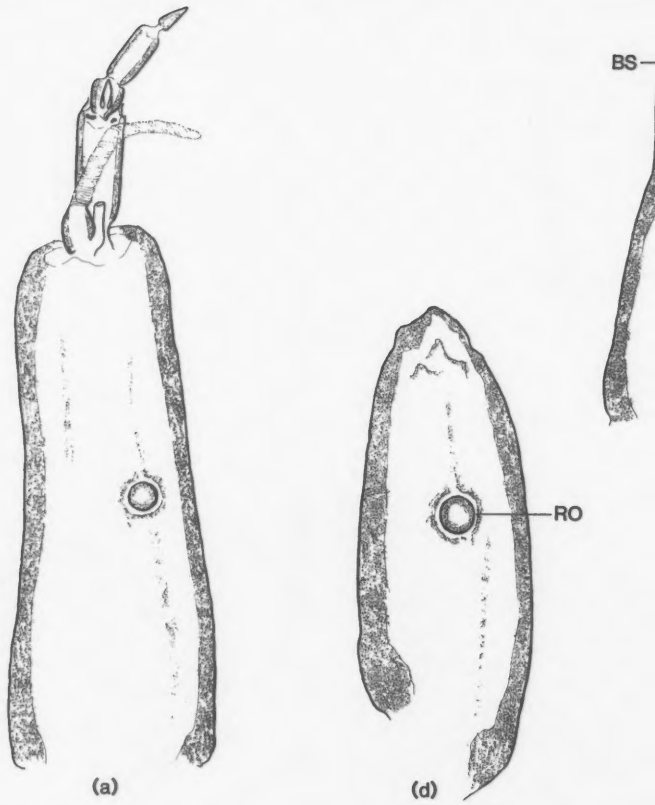
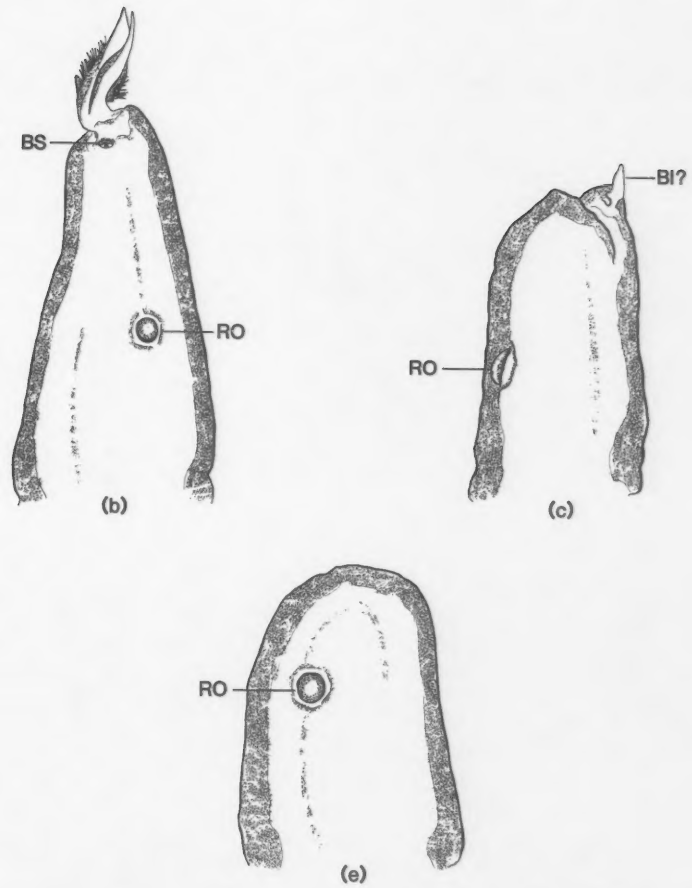


FIG. 8. *Chironomus* spp. category 1(?) antennae with one basal segment bearing no recogn



recognizable receptor(s): (a) normal and (b–e) deformed antennae. Note the "wand" in Fig. 8b.

Basal segments that are entirely devoid of any structures frequently become more heavily sclerotized (Fig. 8) to the point where the apex of the segment is completely sealed over by a heavy chitinous wall (Fig. 8e). The reduction in total length of the antennae is most apparent in these severely deformed specimens. The increasing frequency of unusual or displaced structures is also apparent through Fig. 4–8.

Classification of questionable segments

The presence of questionable segments introduces an element of ambiguity to the interpretation of segmental complement. Those antennae in which classification involves the interpretation of such segments form intermediate categories identified in Table 2 by question marks after the category designation. The rules for classifying questionable segments are outlined below.

(a) Subapically mounted terminal segments — Five-segmented antennae are regarded as questionable when the fifth (terminal) segment is mounted along the side of the fourth segment rather than at its apex. The examples illustrated in Fig. 9b–9d belong in basic category 5(?), that is, four unambiguous segments are present while the fifth is problematic.

(b) Incomplete sclerotization of segment walls — A segment is regarded as questionable if part or all of the segment wall is incompletely sclerotized. Antennae in which the walls of segments three and two are incompletely sclerotized are illustrated in Fig. 9e and 9f, respectively; both examples belong in basic category 5(?). In Fig. 9g, the second antennal segment shows no sign of sclerotization; since three unambiguous segments are present while the fourth (segment 2) is questionable, the antennae is classified in category 4(?).

(c) Clustered apical segments — When more than one apparent segment appears at the apex of the penultimate segment, each is regarded as questionable, but only one is taken into account in the classification. Thus, in Fig. 10b, even though the third segment bears three apparent segments at its apex, the antenna is classified in category 4(?) on the basis of three confirmed segments and one questionable (clustered) segment.

(d) Reduced or stunted segments — Individual segments that are reduced in size but do not affect the total length of the antenna to any degree are also regarded as questionable. The third and fourth segments in Fig. 10c and 10d, respectively, are very small compared with the same segments in normal antennae. The third segment in Fig. 10e similarly is much reduced, but does not appear contiguous with the fourth segment. All are examples of basic category 5(?). The examples in Fig. 10f and 10g also have reduced or stunted segments, but because some of the segments were obscured (Fig. 10f) or lost (Fig. 10g), the antennae cannot be classified with certainty. Antennae that are obviously deformed, but cannot be classified, are labelled "unassigned" and are excluded from the calculation of the Index of Severity of Antennal Deformation (ISAD); such specimens, however, are included in the calculation of the incidence of antennal deformation.

(e) Fused segments — Antennae with partially or incompletely fused segments are classified independently of antennae with completely fused segments. Where two segments are incompletely fused, one is regarded as a full segment, while the second is regarded as questionable. In contrast, where two or more segments are completely fused, the fused structure is regarded as a questionable segment. In Fig. 11b–11d, the second and third antennal segments are partially fused, but because of the progressive loss of apical segments, classifications range through basic categories 5(?), 4(?), and 3(?), respectively. In Fig. 11e and 11f, the segment or segments (?) at the apex of the basal segment are completely fused, with the style mounted laterally along the side of each. Both extremes are classified in basic category 2(?) on the basis of one unambiguous (basal) segment and one questionable (fused) segment.

(f) Multiple questionable segments — Variant segments in antennae where more than one segment displays morphological variation are regarded collectively as a single questionable segment. In Fig. 12b, the subapically mounted terminal segment and the reduced or stunted third segment are regarded as questionable whereas segments one (basal), two, and four are regarded as true segments. By comparison, the antenna in Fig. 12c has a subapically mounted terminal segment and incompletely sclerotized walls on both segments three and four; only segments one and two are regarded as true segments. The two antennae therefore are classified in basic categories 4(?) and 3(?), respectively.

The questionable segment, or segments, in Fig. 12d are fused directly to the apex of the basal segment of the antenna, whereas in Fig. 12e the four (?) apical segments are strongly reduced in size. In both cases, only the basal segment is regarded as a true segment; both antennae, therefore, are classified in basic category 2(?).

(g) Lost or missing segments — Antennae that are obviously deformed in some cases cannot be classified because segments are lost or missing (Fig. 10f and 10g). Antennae with lost or missing segments can readily be distinguished from those whose segments are lacking due to deformation by the presence of at least a portion of the intersegmental articulating membrane which would normally link the two segments. On the deformed antenna in Fig. 12f, this membrane appears as a flap or mound of connective tissue at the apex of the stunted basal segment. The same connective tissue appears in Fig. 12g at the apex of the third segment of an apparently normal antenna. As stated above, deformed antennae with lost or missing segments are listed as "unassigned"; such antennae are excluded from the calculation of ISAD, but are included in the calculation of the incidence of antennal deformation.

(h) Paired antennal deformities — In specimens where both antennae are deformed, the assessment of morphological response is based on the cumulative valuation of both antennae. In Fig. 13, classification of individual antenna ranges from basic categories 2 and 4 in Fig. 13a to 1(?) and 3(?) in Fig. 13d. Based on these classifications, the basic index for these specimens would be 18 and 30, respectively.

Step 2: Reduction in Length of Deformed Antennae

The reduction in length of deformed antennae is ranked on a scale from 1 to 9. The initial 10% loss in length (Table 3) is considered within the range of normal variability between antennal pairs and is discounted; subsequent losses in length, however, are assigned cumulative numerical values of 1 for each successive 10% loss in length. In larvae with only one deformed antenna, the length of the deformed antenna is compared with its normal-sized counterpart. In cases where the normal-sized counterpart is missing, or in cases where both antennae are deformed, the length of the deformed antenna is compared with the average length of antennae on normal larvae of comparable size and type. In the latter case where both antennae are deformed, the estimates are additive, making possible a total of 18 for reduction in antennal length.

The reduction in length of deformed antennae can occur

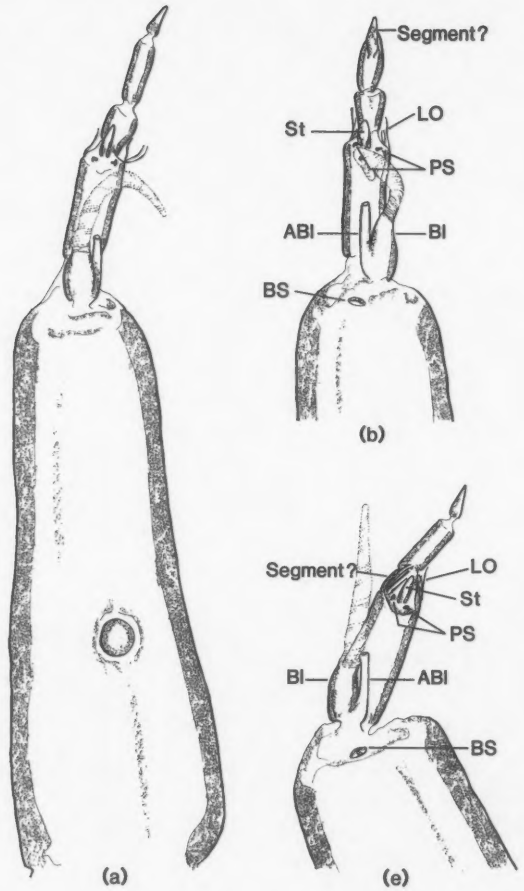
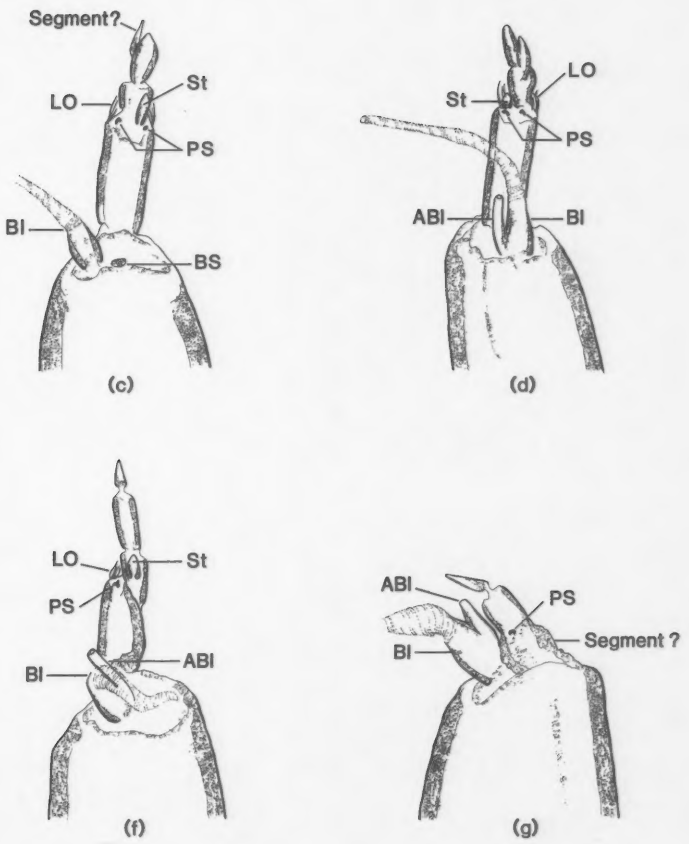


FIG. 9. Variability in *Chironomus* spp. deformed antennae with subapically mounted te
category 5(?), and (g) category 4(?) antennae.



ounted terminal segments (b–d) and segments with incompletely sclerotized walls (e–g): (a) normal, (b–f)

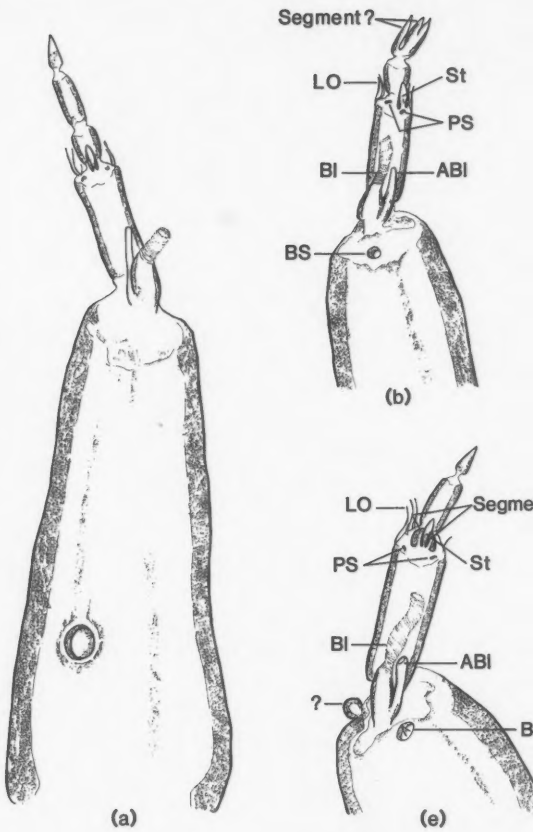
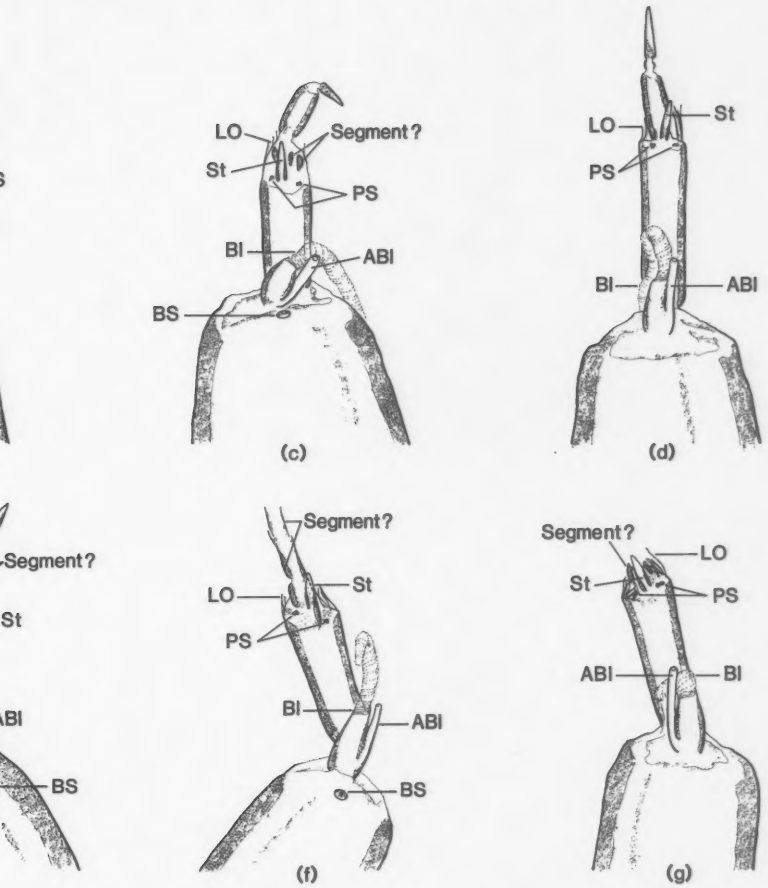


FIG. 10. Variability in *Chironomus* spp. deformed antennae with clustered apical
category 4(?), (c–e) category 5(?), and (f–g) unassigned antennae.



d apical segments (b), reduced or stunted segments (c–e), and missing or obscured segments (f, g): (a) normal, (b)

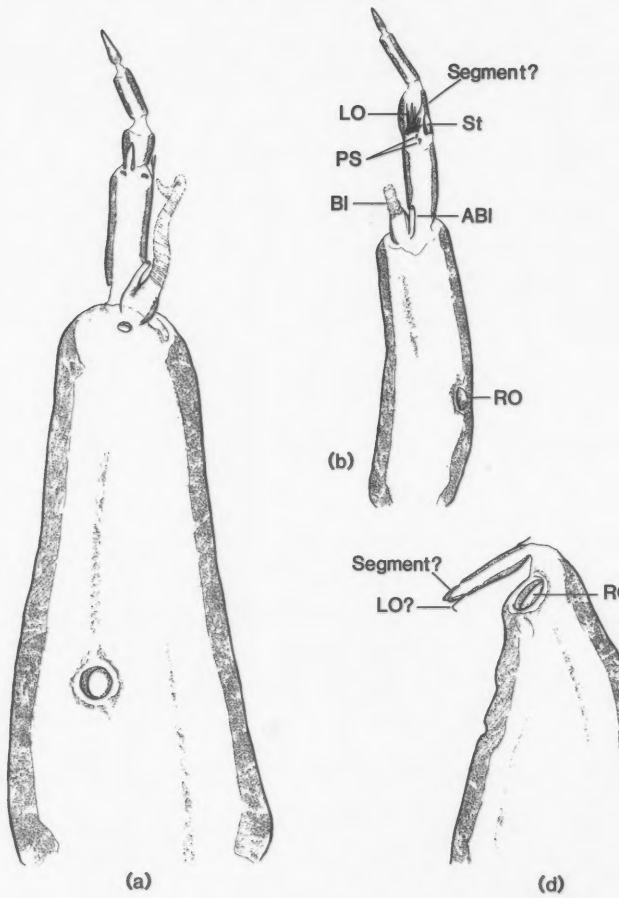
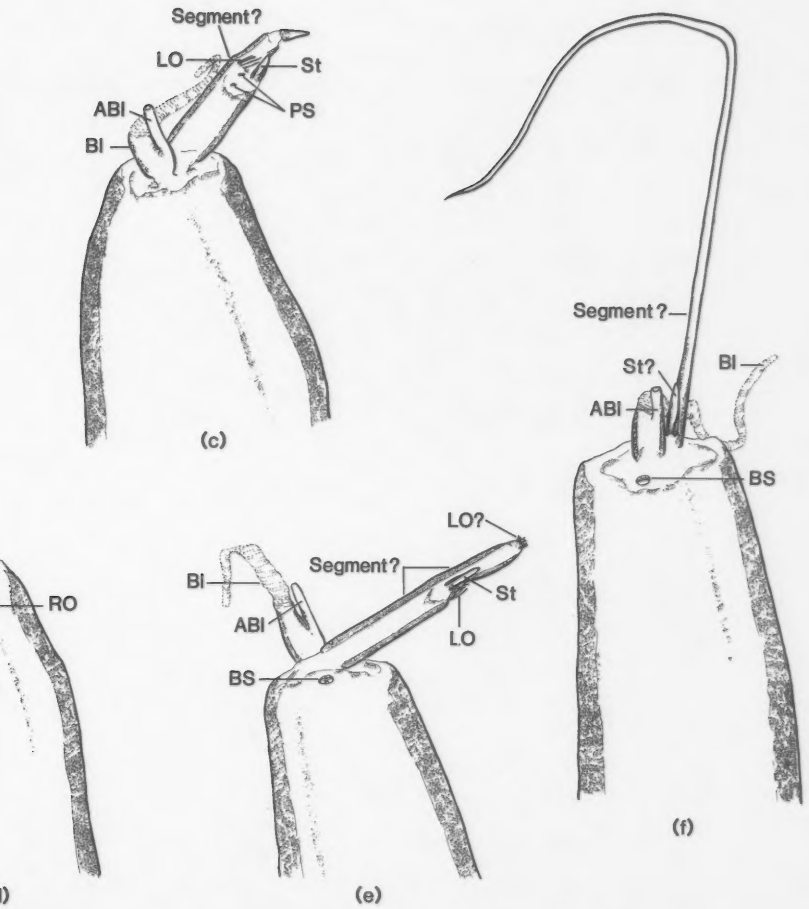


FIG. 11. Variability in *Chironomus* spp. deformed antennae with fused segments:



egments: (a) normal, (b) category 5(?), (c) category 4(?), (d) category 3(?), and (e–f) category 2(?) antennae.

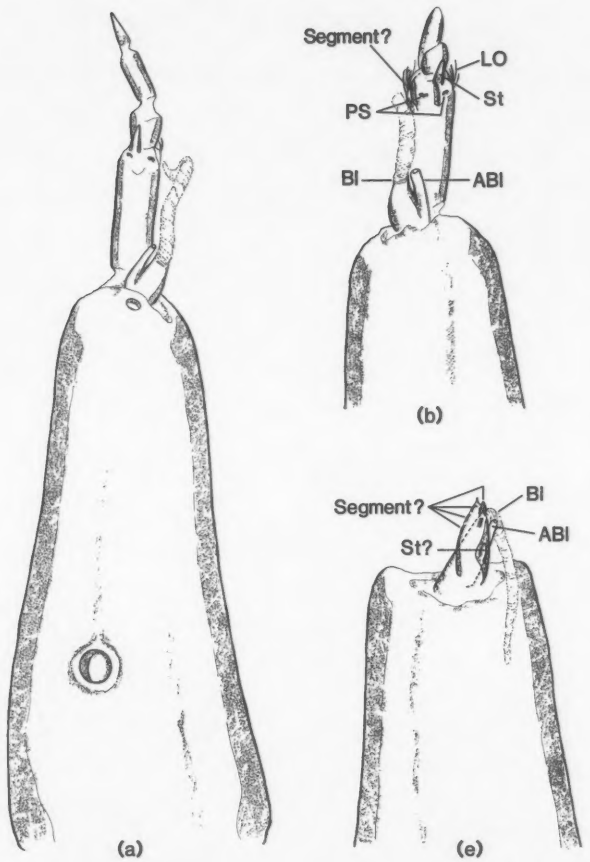
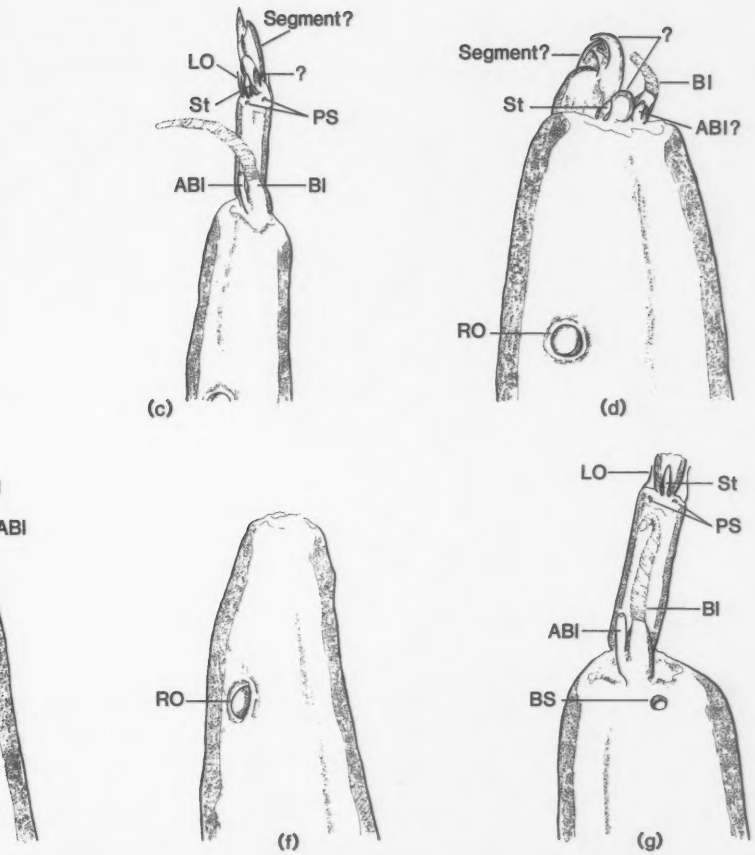


FIG. 12. Variability in *Chironomus* spp. deformed antennae with multiple questionable s
(d–e) category 2(?) antennae. Examples in Fig. 12f and 12g are of antennae with missing



onable segments (b–e) and lost or missing segments (f, g): (a) normal, (b) category 4(?), (c) category 3(?), and
missing segments; note the tell-tale flap of intersegmental connective tissue at each break point.

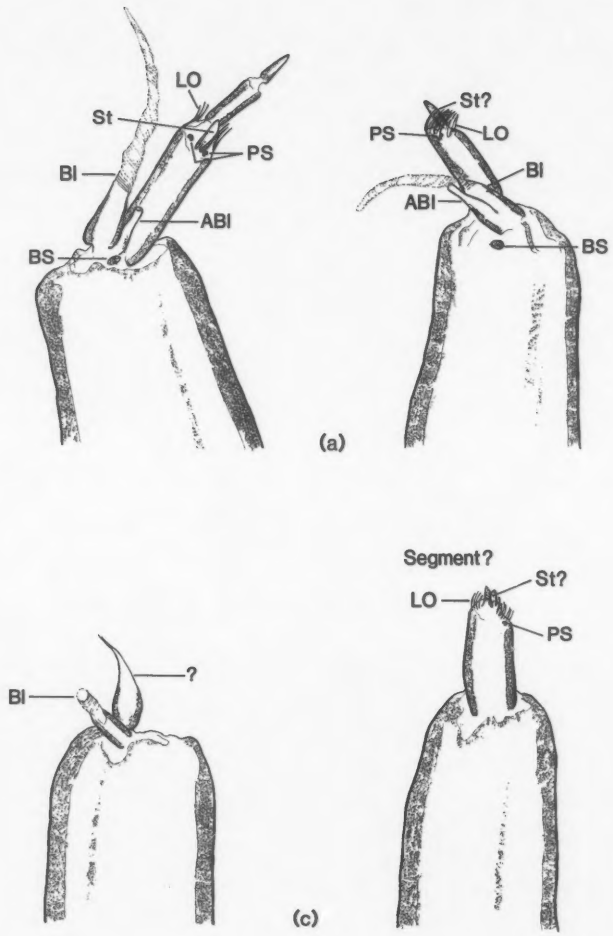
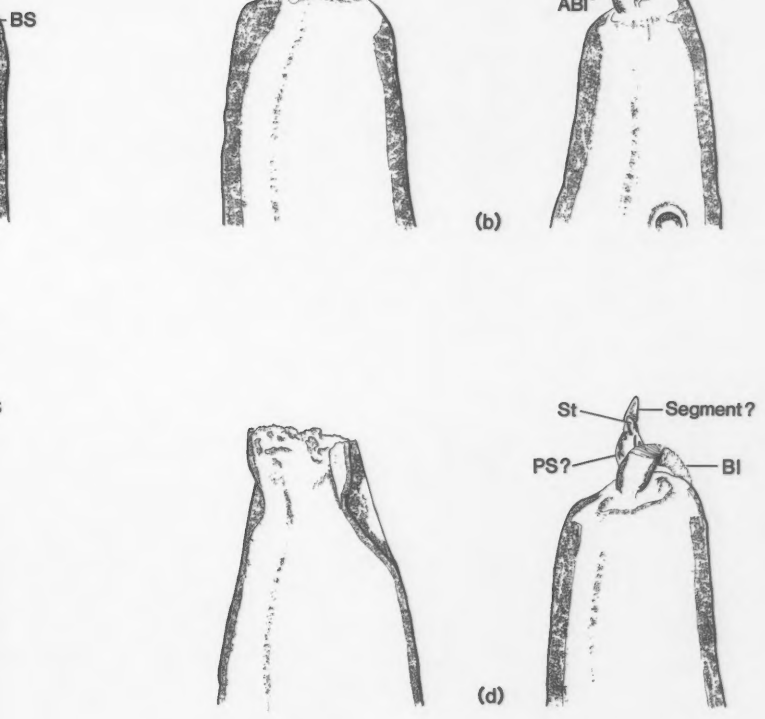


FIG. 13. Variability in *Chironomus* spp. larvae with both antennae deformed: (a) categori
categories 19 and 11 (BIN 30).



categories 5 and 13 (BIN 18), (b) categories 9 and 11 (BIN 20), (c) categories 17 and 11 (BIN 28), and (d)

generally or be confined to either basal or distal segments. Although the antennae shown in Fig. 14 all belong in basic category 5, the range of variation in the length of the basal segment is obvious. The basal segment of the antenna in Fig. 14b is noticeably shorter than that of its normal counterpart. The four distal segments, however, are longer and almost compensate for the shorter basal segment. As a result, the loss in total length overall is only 1.2%, so no value would be accorded the difference in length. In Fig. 14c, both basal and distal segments are reduced in length compared with the normal counterpart; the reduction in length of 37.2% would therefore be assigned a factor of 3. The difference in length of the antenna in Fig. 14d is primarily in the basal segment; in this case the difference of 43.9% would be assigned a factor of 4.

The reductions in length of the deformed antennae in Fig. 15 occur primarily in the distal segments. In Fig. 15b, the shortened basal segment is compensated for by an elongated fourth segment whereas in Fig. 15c shorter distal segments, particularly the fourth segment, compensate for an elongated basal segment. The respective differences in length are only 4.9 and 7.5% overall, so no points would be assigned from Table 3. The reduction in length in Fig. 15d is confined almost entirely to the distal segments whereas both the distal and, to a lesser degree, the basal segments are reduced in length in Fig. 15e. Reductions in the length of these antennae of 11.3 and 29.1% would be assigned factors of 1 and 2, respectively.

In cases where both antennae are deformed, the reduction in length is calculated by comparing the lengths of each deformed antenna with the average length of antennae from normal larvae of comparable size and type (Table 4). For this purpose, comparability of size is determined on the basis of mentum width. For the example in Fig. 13b, the width of the mentum of the deformed specimen was 235.83 μm. Normal larvae taken from three nearby sites in Tobin Lake had average mentum widths of 235.55 μm (range 235.05–238.73 μm) (W. F. Warwick, unpubl. data). The average antennal length for these larvae ($n = 22$) was 213.10 μm. Using these data, the reduction in length of the right- and left-hand antenna was estimated at 32.9 and 25.3%, respectively. The cumulative total for the reduction in antennal length therefore was 58.2% for a length reduction factor of 5.

Step 3: Displacement or Loss of Ring Organ

The loss of the ring organ can occur (Fig. 16) in fully formed, apparently normal antenna and, although not presently confirmed, can probably occur in any basic category of deformed antennae. Antennae lacking the ring organ are assigned a value of 2; in cases where the ring organ has been displaced or its presence is questionable (Fig. 17), a more conservative value of 1 is assigned (Table 5). The displacement of the ring organ generally is toward the apex of the basal segment from the normal lower-middle position. Displacement becomes more apparent as the stunting of the basal segment becomes more extreme. The antenna in Fig. 17g is unusual in that the segment is nearly full size and yet the ring organ (RO?) is located directly on the apex of the segment.

The presence or absence of the basiconic sensillum is very difficult to confirm reliably with the light microscope and therefore is not considered here.

Step 4: Apical Fusing of Basal Segments

The fusing or sclerotization of the apex of the basal segment is a feature of more seriously deformed and stunted antennae. Although there are specimens in basic category 1(?) that do not display apical fusing (see Fig. 8d or 13d), those that do (Fig. 18) are assigned a value of 2 in recognition of their greater morphological response to stress.

TABLE 3. Scale for indexing the severity of reduction in length of deformed antennae in *Chironomus* spp. larvae.

Loss of length (%)	Length reduction factor
0–10	0
10–20	1
20–30	2
30–40	3
40–50	4
50–60	5
60–70	6
70–80	7
80–90	8
90–100	9

Step 5: Displacement of Sensory Organs

Sensory receptors appended to antennal segments are subject to displacement, modification, or a combination of both effects. The style normally located on the intersegmental articulating membrane connecting the second and third segments has been displaced downwards on to the side of the second segment in Fig. 19b–19e, and that in Fig. 19f to the apex of the basal segment. In Fig. 19g, an appendage believed to be the style appears subapically on the wall of the basal segment; although heavy walled like the style, its exact identity is not known.

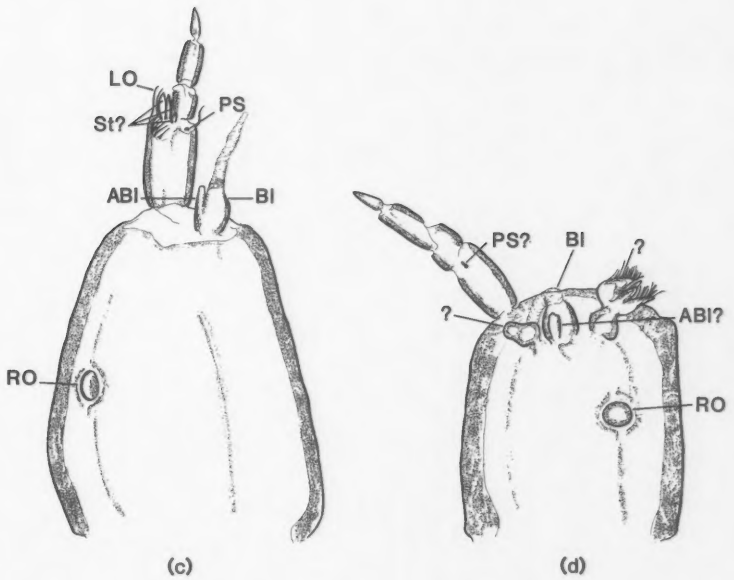
The blade and accessory blade normally located on the intersegmental articulating membrane between the first (basal) and second segments can also be displaced. In Fig. 20b, the blade/accessory blade complex has been displaced upward on to the sclerotized wall of the second segment. In Fig. 20b, the same complex has been displaced downward onto the side of the sclerotized wall of the basal segment. The displacements of the style and the blade/accessory blade complex are both assigned values of 2 as a measure of morphological response to stress.

Step 6: Structures of Unknown Homology

Several antennae have been found bearing trident- (Fig. 20d) or palmate-shaped (Fig. 20e, 20f) structures. The specimens bearing these structures came from Tobin Lake, the first from Site VI and the other two from Site V. Although their homology has not been ascertained, the fact that they were sampled from different sites in Tobin Lake suggests that some specificity of response may be involved in their formation.

Other, less-structured appurtenances, referred to euphemistically as "warts" and "wands," appear on many, more seriously deformed antennae. Good examples of warts appear on the antennae in Fig. 10e, 12d, and 20d; good examples of wands appear in Fig. 8b and 17e. In Table 5, highly structured

FIG. 14. Variability in *Chironomus* spp. category 5 antennae showing redu



ving reduction in length of basal segment: (a) normal and (b–d) deformed antennae.

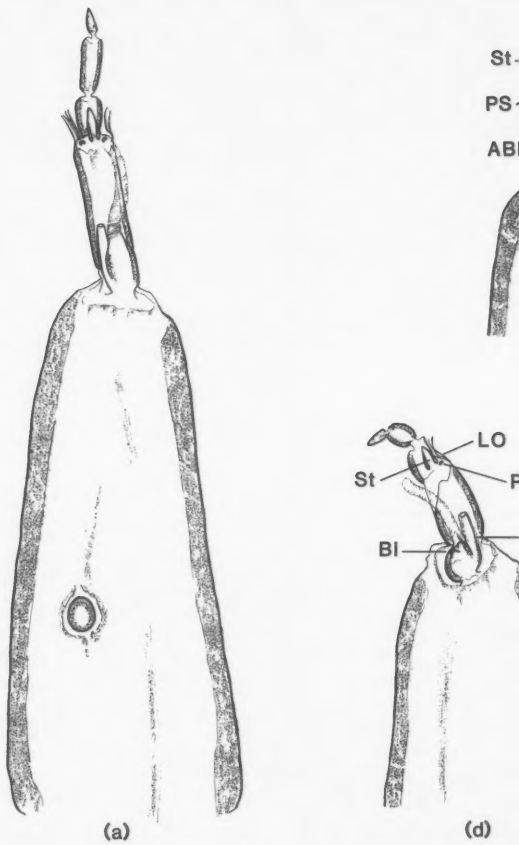
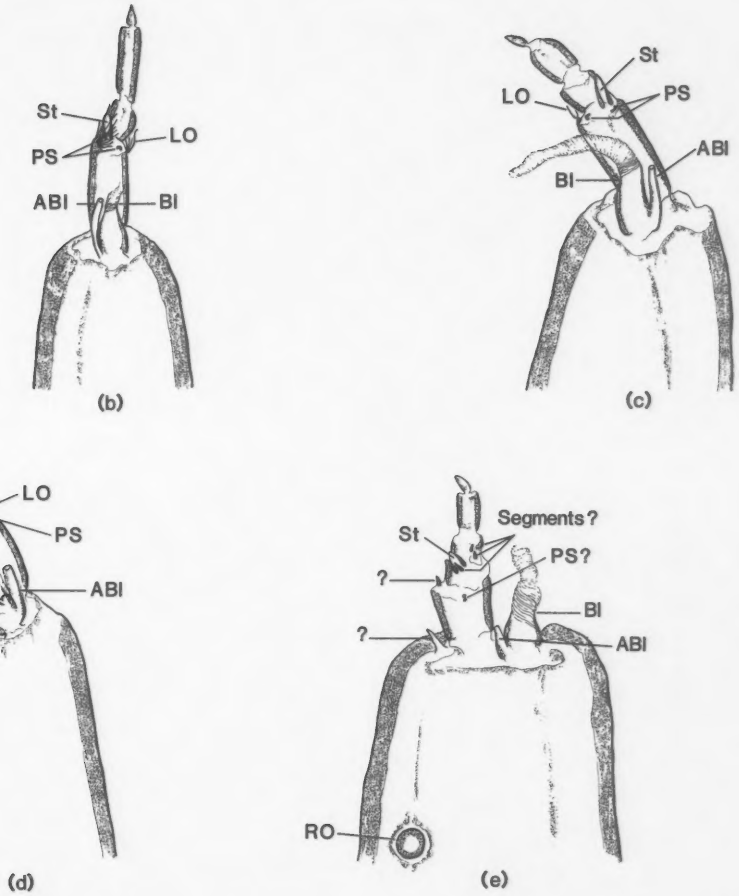


FIG. 15. Variability in *Chironomus* spp. category 5 antennae showing reduction in len
unknown homology in Fig. 15e.



on in lengths of distal segments: (a) normal and (b–e) deformed antennae. Note the number of minor structures of

TABLE 4. Calculation of the length reduction factor for *Chironomus anthracinus* type larvae with both antennae deformed (all measurements in micrometres).

Parameter	Measurement	Figure reference 13b	13d	16f
Deformed specimens	Mentum width	235.83	245.53	241.24
	Antennal length (left)	159.19	140.84	110.68
	Antennal length (right)	143.00	140.62	68.93
Normal specimens	Mentum width (mean)	235.55	245.57	241.50
	Mentum width (range)	235.05–238.73	241.02–249.52	238.38–245.30
	Antennal length (mean)	213.10	218.35	217.45
	Number of specimens (*n*)	22	20	22
Length reduction (%)	Antenna (left)	25.3	35.5	49.1
	Antenna (right)	32.9	35.6	68.3
Total length reduction (%)		58.2	71.1	117.4
Length reduction factor		5	7	11

appendages of unknown homology are assigned a value of 2, while less-structured appurtenances are given a value of 1.

Calculation of the Index of Morphological Response

An Index of Morphological Response (IMR) is calculated for each deformed antenna or pair of deformed antennae according to the six basic steps summarized in Table 5. Examples of these calculations are presented in Table 6 for specimens selected from Fig. 3 to 20. Note that in many cases (for example IMR = 18), individual estimates of the severity of antennal deformation can be derived from a number of different antennal configurations. In cases where both antennae were deformed, IMR is the sum for both antennae.

Calculations of the Index of Severity of Antennal Deformation

The ISAD is interpreted as a measure of the morphological response to stress exhibited by a population of chironomid larvae exposed to toxicants in the aquatic environment. The index is defined simply as

$$\text{ISAD} = \frac{\Sigma\,\text{IMR}}{n}$$

where IMR represents the total of the indices of morphological response describing the degree of severity of deformation in individual antenna and n is the total number of specimens examined.

TABLE 5. Summary of the values assigned to individual types of deformities in antennae of *Chironomus* spp. larvae.

Step	Feature	Indices of morphological response (IMR)
1	Basic classification categories	
	(a) Loss of genuine segments	1, 5, 9, 13, 17, and 21
	(b) Presence of questionable segments	3, 7, 11, 15, and 19
2	Reduction in antennal length	1–9
3	Displacement or loss of ring organ	1–2
4	Apical fusing of basal segments	2
5	Displacement of sensory organs	2
6	Structures of unknown homology	
	(a) Major structures	2
	(b) Minor structures	1

Deformed Antennae in Control and Natural Populations of *Chironomus*

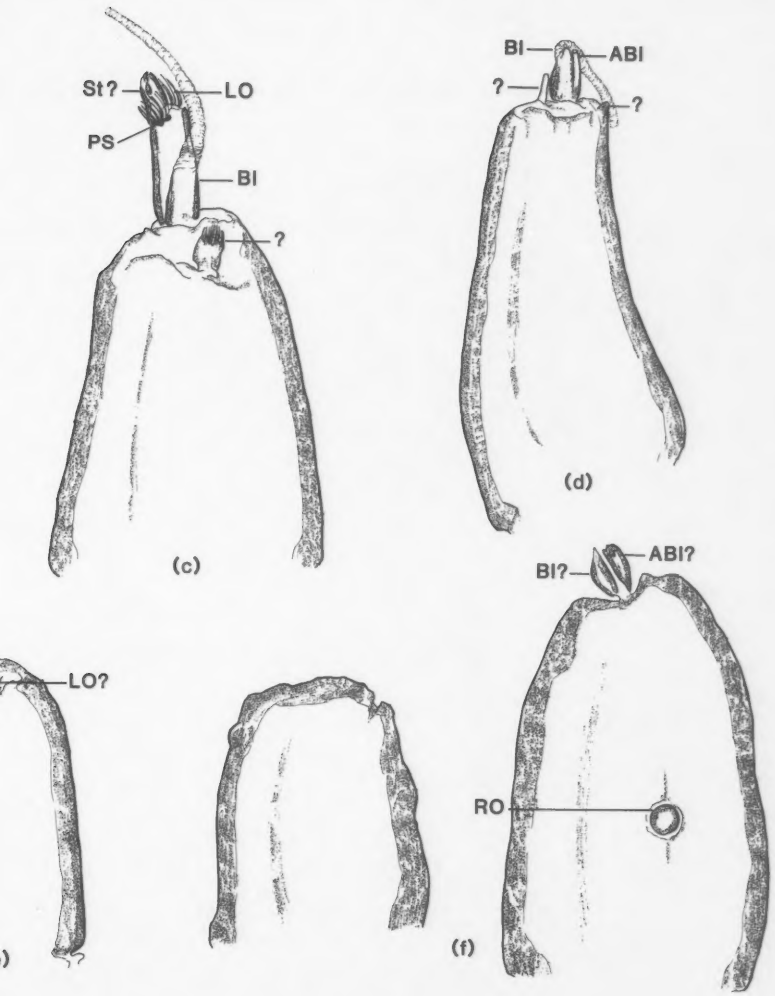
Background Occurrences

Morphological deformities do occur in the antennae of natural populations of *Chironomus* living under essentially ideal environmental conditions. Unfortunately the data do not exist to provide background information either on the incidence or severity of antennal deformations that might be expected in unstressed field populations. Previously, because they occurred only rarely, or at least were rarely noted, deformities were regarded more from the point of curiosity than as an indication of environmental sensitivity. Even today, with the growing awareness of the sensitivity of chironomid larvae and other members of the benthic community to toxic stress, there has been no satisfactory framework within which to quantify their response to chemical contaminants in the environment. This has been particularly true with regard to antennal structures where traditional methods of preparation and mounting have precluded detailed, systematic scrutinization of morphological structures. The only comparable data available for direct comparison (Table 7) are specimens from master cultures of *Chironomus tentans* Fab. sampled by Hamilton and Saether (1971) as experimental controls. These cultures were maintained under stringent conditions in five different laboratories in the Freshwater Institute, Winnipeg, Manitoba, during the early 1970's (Uthe 1972). The specimens were remounted as described above and only one deformity — an abnormal premandible with a single rather than bifid tooth — was found in the 78 larvae examined; no deformed antennae were found.

Occurrences in Natural Populations

The incidence and severity of antennal deformations in *Chironomus* fauna from comparatively uncontaminated Last

FIG. 16. Variability in *Chironomus* spp. antennae w



tennae with ring organ lacking: (a) normal and (b–f) deformed antennae.

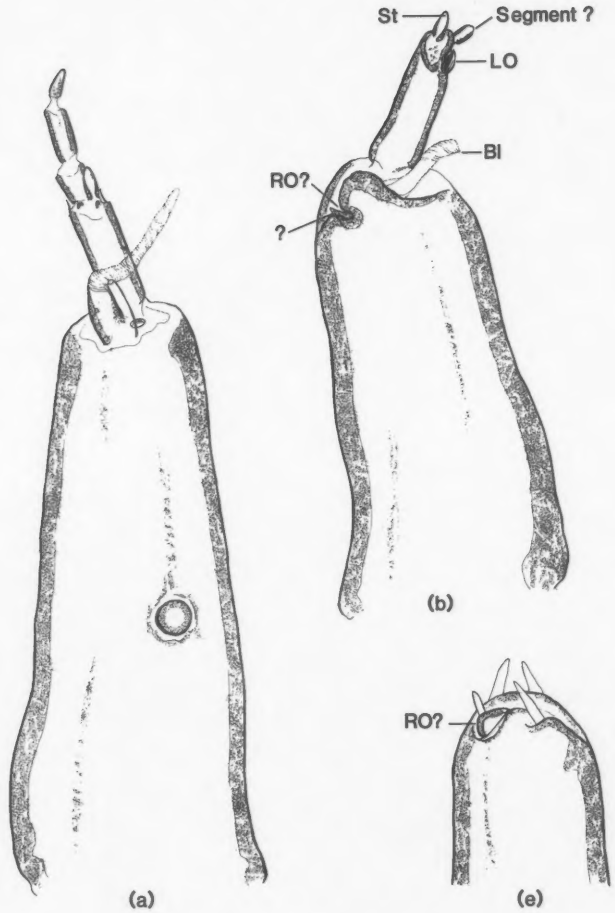
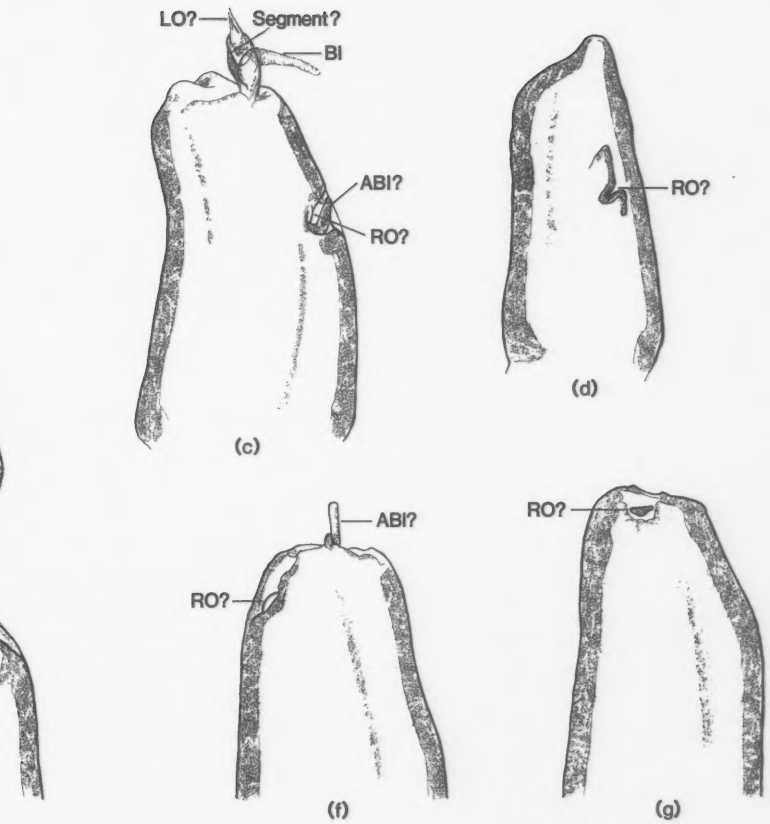


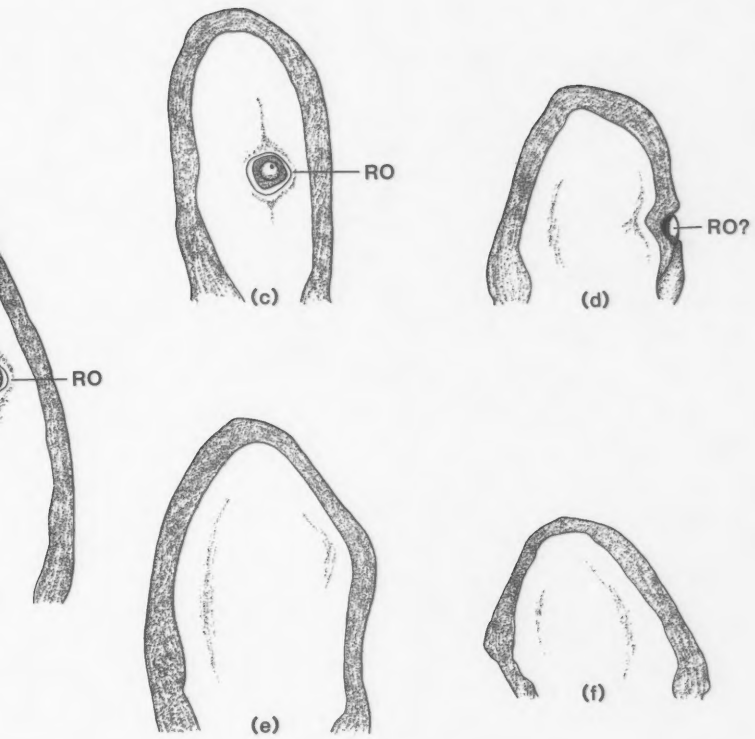
FIG. 17. Variability in *Chironomus* spp. antennae in which pres

nt ?



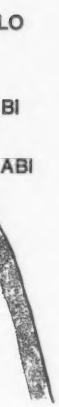
hich presence of ring organ is questionable: (a) normal and (b–g) deformed antennae.

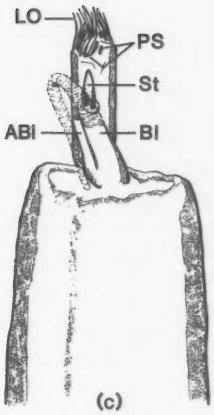
FIG. 18. Variability in *Chironomus* spp. antennae

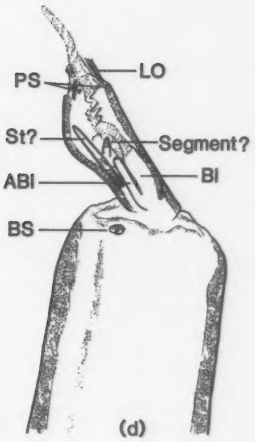


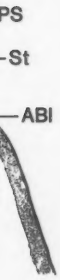
antennae showing apical fusing: (a) normal and (b–f) deformed antennae.

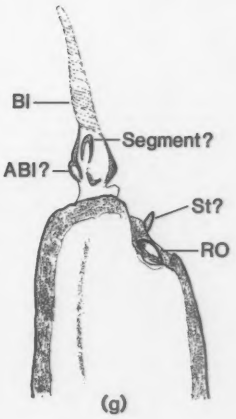
FIG. 19. Variability in *Chironomus* spp. antennae with











nae with laterally mounted styles: (a) normal and (b–g) deformed antennae.

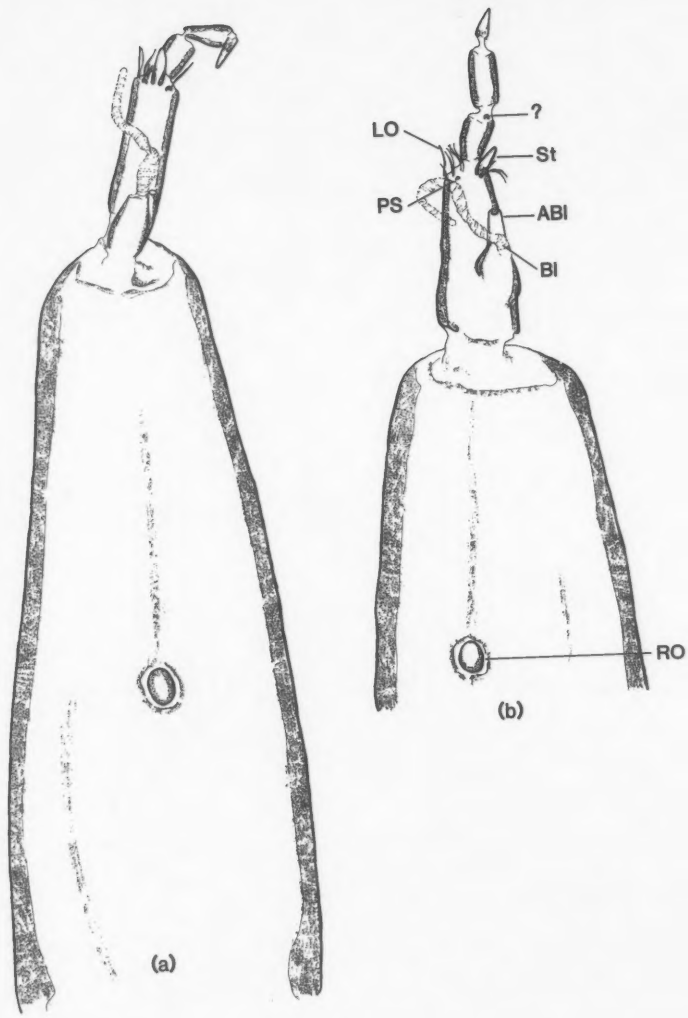
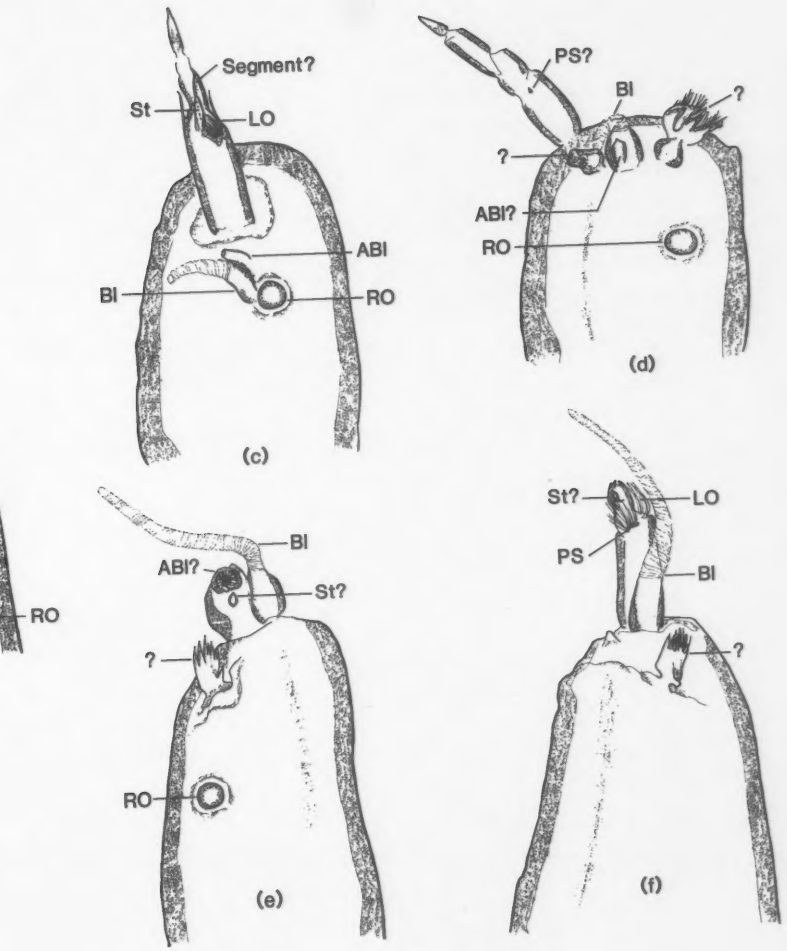


FIG. 20. Variability in *Chironomus* spp. antennae with laterally mounted blades (b, c) and tride
antennae.



and trident- (d) or palmate-shaped structures of unknown homology (e–f): (a) normal and (b–f) deformed

TABLE 6. Examples of estimating the index of morphological response for specimens selected from text Fig. 3–20.

Figure	Basic index number	Length reduction factor	Ring organ	Apical fusing	Displaced sensory organs	Structures of unknown homology		Other	Index of morphological response
						Major	Minor		
14b	1	—	—	—	—	—	—		1
3b	1	1	—	—	—	—	—		2
10c	3	—	—	—	—	—	—		3
9b	3	—	—	—	—	—	—		3
9c	3	1	—	—	—	—	—		4
3c	5	—	—	—	—	—	—		5
16b	1	2	2	—	—	—	—		5
4c	5	1	—	—	—	—	—		6
14c	1	3	—	—	—	—	2		6
10b	7	—	—	—	—	—	—		7
4d	5	3	—	—	—	—	—		8
14d	1	4	—	—	—	2	1		8
5b	9	2	—	—	—	—	—		11
17b	11	2	1	—	—	—	—		14
6d	13	2	—	—	—	—	—		15
6e	13	3	—	—	—	—	—		16
19c	13	1	—	—	2	—	—		16
19d	11	1	—	—	4	—	—		16
12e	15	3	—	—	—	—	—		18
19f	15	1	—	—	2	—	—		18
16c(20f)	9	3	2	—	2	2	—		18
7e	17	5	—	—	—	—	—		22
7c	17	4	—	—	2	—	—		23
12d	15	4	—	—	2	—	2		23
19g	15	4	1	—	4	—	—		24
20e	17	4	—	—	—	2	2		25
8b	19	5	—	—	—	—	2		26
17f	19	5	1	—	—	—	1		26
13b	9	2	—	—	—	—	1		26
	11	3	—	—	—	—	—		
3g	19	5	—	4	—	—	1		29
17d	19	5	1	4	—	—	—		29
8e	19	5	1	4	—	—	—		29
18d	19	7	1	4	—	—	—		31
18f	19	7	2	4	—	—	—		32
17e	19	5	1	4	—	—	4		33
13d	11	3	—	—	—	—	—		37
	19	4	—	—	—	—	—		
16f	19	5	—	—	—	—	—		55
	19	6	2	4	—	—	—		

Mountain Lake and contaminated Tobin Lake sites are compared in Table 8. Variability in ISAD between duplicate samples was compared using Cochran's modified *t*-test for dissimilar variances (Dixon and Massey 1969). The *t*-tests indicate that the differences between duplicate samples were not significant. The appropriate values were $t = 1.60$ ($t_{0.05} = 1.960$ for 501 df) for Site II, $t = 1.03$ ($t_{0.05} = 1.960$ for 685 df) for Site V, and $t = 0.71$ ($t_{0.05} = 1.960$ for 473 df) for Site VI. These results show that the severity of antennal deformation is consistent between duplicate samples and that the data can be pooled for intersite comparisons.

Although the incidence of antennal deformation differs markedly in Table 8 between (comparatively) uncontaminated and contaminated sites, ISAD values do not vary as widely as might be expected, particularly between the two Tobin Lake sites. A *t*-test between Tobin Lake Sites V and VI using combined duplicate data sets shows no significant difference ($t = 1.30$, $t_{0.05} = 1.960$ for 1088 df) between the two sites.

The differences in the patterns of morphological response between individual sites become more apparent, however, when the distribution of rankings within ISAD are grouped into classes of morphological response and the number of antennae within each class are expressed in terms of numbers per 1000 population (Fig. 21). In Last Mountain Lake, although the severity of deformities in *Chironomus* antennae were comparatively mild and few in number, a small number were reasonably severely deformed. This seems to indicate that either the tendency for malformation in physiologically weaker individuals can span the entire potential range of morphological variation or the effects of suspected contaminant residues in the more northern areas of Last Mountain Lake may be greater than expected.

TABLE 7. Incidence of deformities in larvae reared under control conditions in the Freshwater Institute, Winnipeg, Manitoba.

Culture	Number of larvae examined	Number of larvae deformed
Hamilton	18	1[a]
Yurkowski	19	—
Clayton	9	—
Flannagan	14	—
Master culture (glass)	18	—
Total	78	1[a]

[a]Abnormal premandible.

Compared with Last Mountain Lake, the Tobin Lake sites displayed (Fig. 21) a far greater range of variability in deformed antennae, both in terms of numbers and degree of severity. Even between the two Tobin Lake sites, considerable variability is apparent. At Site V, the range of morphological response was closely grouped between 1 and 40. The concentration of deformed antennae in IMR classes 21–25 skewed the frequency distribution slightly to the right. At Site VI, the range of morphological response was bimodal, with both modes strongly skewed to the left. The greater number of deformed antennae at this site fell in IMR classes 1–5; the most severely deformed antennae occurred in IMR classes 51–55. Comparison of the three sites in Fig. 21 clearly shows that the frequency of response must be taken into account in the interpretation of ISAD and its relevance to contaminant stress.

Chironomus Larvae Exposed to DDE

Hamilton and Saether (1971) exposed larvae of *Chironomus tentans* Fab. to a range of concentrations of DDE in an attempt to experimentally reproduce deformed individuals similar to those found in Lake Erie and the Okanagan lakes. One severely deformed larva was noted, but generally they were unable to determine any dose–response relationship. Their analyses did not include antennal features, probably because the traditional methods used to prepare and mount their specimens precluded the level of detail required for such an analysis.

The specimens from these pilot studies were remounted and reexamined in detail. Although the data are based on very small sample populations (Table 9), the results show (Fig. 22) that the incidence of antennal deformation decreased as the concentrations of DDE increased. Linear regression analysis indicates that the inverse relationship was highly significant ($r = 0.971$, $r_{0.01} = 0.959$ for 3 df). The same data set shows (Fig. 22) that ISAD also decreased as the concentration of DDE increased. In this case, the inverse relationship was significant between 95 and 99% probability ($r = 0.946$, $r_{0.05} = 0.878$). These dose–response relationships provide the first clear indication that morphological changes are induced in proportion to the concentration of a specific contaminant.

The inverse relationship indicated by both indices was not wholly anticipated. One possible explanation is that the relationship represents an isolated portion of the total response range of antennae to DDE. Quantal dose–response relationships can be regarded as a graded response with the well-known "bell-shaped" population distribution if, as in ISAD, the entire population is considered as an individual (Chan et al. 1984). In this case, the range of DDE concentrations used may only span a range of antennal response in which resistance to DDE is increasing. Resistance possibly could be exerted through selective survival of the more resistant members of the cohort, but unfortunately no measures of mortality or population size were kept from these pilot studies. If this hypothesis is correct, the range of greatest antennal sensitivity probably is somewhat less than 1 μg DDE·L^{-1} or its chemical equivalent.

Another possibility is that the relationship represents the limitations imposed on single generation dose–response experiments where sufficient time is not available for full morphological response to develop. Alternatively, other information from this data set indicates that deformation in the antennae is replaced at higher concentration levels by deformation in other, more heavily sclerotized, less sensitive morphological structures, notably the teeth of the mentum and mandibles (Warwick 1985). Possibly the ability of sensitive antennae to react may rapidly become saturated at higher concentrations of contaminant.

Comparisons Between Differentially Stressed Lake Ecosystems

The average incidence of deformed antennae in Tobin Lake (Table 10) was almost 6.5 times higher than in Last Mountain Lake. Tobin Lake receives via the Saskatchewan River system industrial effluents from a number of western Canadian cities and toxic residues from the extensive agricultural areas of the western plains. In contrast, the northern sector of Last Mountain Lake receives little or no industrial contamination and probably

TABLE 8. Comparison of the incidence (%) and severity (ISAD) of antennal deformation in *Chironomus* spp. larvae in duplicate samples from Last Mountain Lake (comparatively uncontaminated) and Tobin Lake (contaminated) sites.

	Last Mountain Lake		Tobin Lake			
Sample designation	IIa	IIb	Va	Vb	VIa	VIb
Number of larvae examined	459	476	340	350	231	257
Number of *Chironomus* larvae	419	418	338	350	231	257
Number of deformed antennae	8	2	25	33	16	18
Incidence of deformed antennae (%)	1.9	0.5	7.4	9.4	6.9	7.0
Number of evaluated antennae (d)	7	2	25	33	16	18
Total IMR	82	17	473	653	232	350
ISAD	0.20	0.04	1.40	1.87	1.00	1.36

TABLE 9. Incidence (%) and severity (ISAD) of antennal deformation in larvae of *Chironomus tentans* Fab. exposed to different concentrations of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene (DDE).

	DDE ($\mu g \cdot L^{-1}$)				
	1	5	10	15	20
Incidence of deformed antennae (%)	25.0	17.1	12.7	7.1	4.6
Number of larvae examined	4	35	55	28	131
Number of deformed antennae	1	6	7	2	6
Number of evaluated antennae (*d*)	1	6	6	2	6
Total IMR	5	38	43	10	54
ISAD	1.25	1.09	0.78	0.36	0.41
Confidence limits (95%: ISAD)	±3.98	±0.90	±0.72	±0.51	±0.37

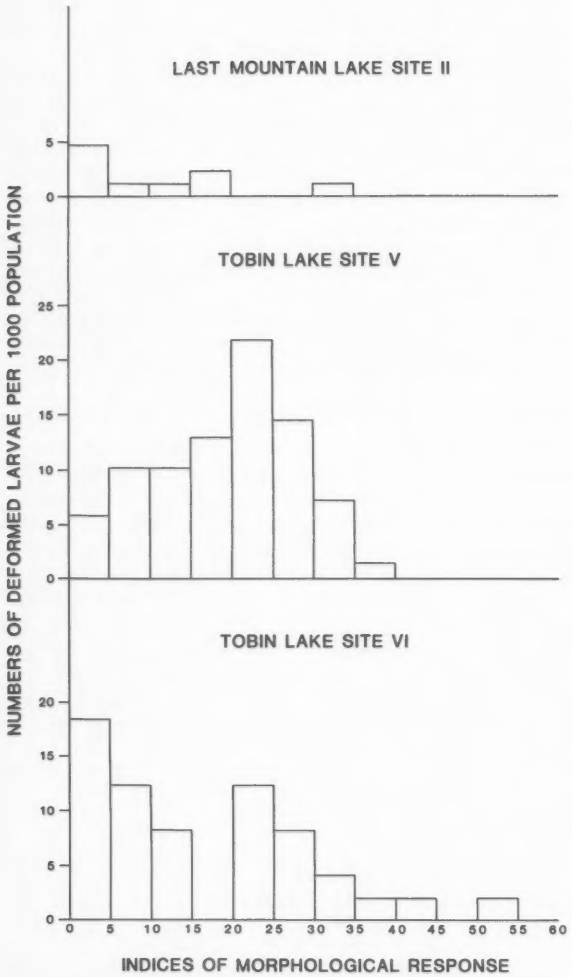

LAST MOUNTAIN LAKE SITE II
TOBIN LAKE SITE V
TOBIN LAKE SITE VI
NUMBERS OF DEFORMED LARVAE PER 1000 POPULATION
INDICES OF MORPHOLOGICAL RESPONSE

FIG. 21. Indices of morphological response by class for Last Mountain Lake and Tobin Lake Sites V and VI.

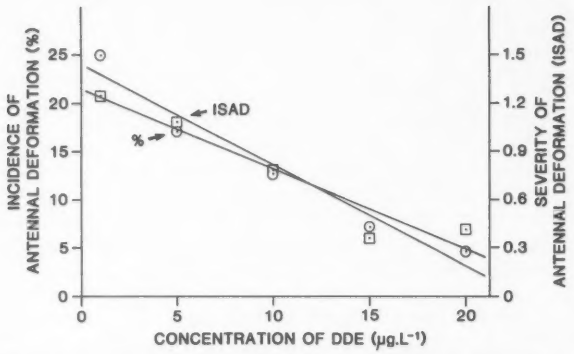


FIG. 22. Dose–response relationships between the incidence (%), severity (ISAD), and concentration ($\mu g \cdot L^{-1}$) in *Chironomus tentans* Fab. larvae exposed to DDE. Regression equation for incidence of deformation is $y = 2401 - 1.05x$. Regression equation for ISAD is $y = 1.29 - 0.05x$.

little toxic residue from agricultural sources. The presence of agricultural contaminants cannot be discounted entirely, however, because the lake is located in an area of extensive agriculture. Be that as it may, the impact of the contaminant differential between the (comparatively) uncontaminated Last Mountain Lake site and the contaminated Tobin Lake sites is clearly evident in the difference in the frequencies of deformed antennae in *Chironomus* larvae from the two lakes.

The incidence of deformed antennae in the Tobin Lake fauna (Table 10) declined from 8.43% at Site V to 6.97% at Site VI. The decline occurred in the direction of the hydraulic gradient established between the inflow of the Saskatchewan River and the Squaw Rapids dam. The ISAD showed the same pattern; ISAD estimates for Site V averaged 1.64 compared with 1.19 at Site VI. Reservoir hydraulics are such that energy dissipation between the inflow and the dam should establish a pattern of selective deposition along the energy gradient. The separation of contaminant-laden particles should occur not only according to particle size, but also according to chemical species. Samoiloff et al. (1983) found that of 52 potentially toxic compounds isolated from the sediments of Tobin Lake Sites VII and VIII, only 5 were common to both sites (Table 11). These data

TABLE 10. Comparison of the incidence (%) and severity (ISAD) of antennal deformations in *Chironomus* spp. larvae from Last Mountain Lake (comparatively uncontaminated) and Tobin Lake (contaminated) sites.

Site	Incidence of deformed antennae	Severity of antennal deformation
Last Mountain Lake B	1.19	0.12±0.10
Tobin Lake	8.43	1.64±0.45
Tobin Lake VI	6.97	1.19±0.50

indicate that the hydraulic parameters in the reservoir are operating to produce a chemical gradient as predicted. Sites VII and VIII are located farther apart than Sites V and VI (Fig. 1), but the chironomid fauna at the latter sites is probably exposed to a similar gradient of chemical compounds.

The incidence and severity of antennal deformations show that the toxicological response of the fauna is different and orientated along the gradient of hydraulic energy. The higher values in both indices at Site V initially suggest that the greater chemical stress occurs toward the source of input, that is, the inflow from the Saskatchewan River. However, the experiments with DDE indicate that both indices decrease as the concentration of contaminant increases. Applied to Tobin Lake, this relationship would suggest that the greater concentration of contaminants is to be expected not towards the inflow, but toward the dam site. Although antennal deformities in general are less severe at Site VI than at Site V, the most severe examples of antennal deformation were found at Site VI. Recently, massively deformed larvae were found at a site located between Site VI and the Squaw Rapids dam (W. F. Warwick, unpubl. data). The pattern in Tobin Lake therefore seems to confirm the relationship predicted from the experimental results. Continuing studies are in progress to see if the shift toward more severe deformation in the heavily sclerotized structures of the head capsule predicted from the DDE experiments occurs between Sites V and VI.

Deformed Antennae as a Biological Screening Tool

Deformities and Contaminants

Morphological deformities in chironomid larvae provide a potentially important barometer for monitoring the health of aquatic ecosystems. Because the greater part of their life history is spent in the surficial sediments, chironomid larvae are directly exposed to the "chemical soup" introduced into aquatic environments and, perforce, must integrate the synergistic/antagonistic effects of chemical interactions and incorporate the degradation products from chemical decomposition. By force of circumstance, chironomid larvae directly monitor the net toxic burden of contamination in a manner unmatched by modern chemistry.

Although the linkages between morphological deformities and contaminants presently are based on circumstantial evidence and only limited experimental data, they are compatible with the theory that deformities represent responses to toxic substances introduced into the aquatic environment by man. These substances may include industrial wastes, agricultural pesticides or herbicides, trace metals, and radionuclides.

Factors other than contaminants, such as salinity, temperature, oxygen depletion, changes in water depth, and the erosion and deposition of mineral sediments, could conceivably cause sufficient stress to induce deformities. Under normal circumstances, changes in these factors probably would initiate successional changes in community structure and not effect morphological response. Saether (1970) suggested that deformities may occur in individuals living near the limits of their ecological range, but largely discounted the idea because deformed larvae had never before been reported in the literature. Hare and Carter (1976) also reported that deformed larvae in Parry Sound were not ecologically limited in the classical sense, but were near the limit of their "industrial effluent tolerance range," because all deformed specimens were found near areas where the intensity of industrial and commercial activity was greatest. In order to induce deformities, changes in natural factors would have to occur at such a rate that the normal processes of ecological succession are abrogated or rendered ineffectual. Under such circumstances, morphological deformities might be induced in communities exposed to the stress of rapid ecological change.

Basic limnological factors could also affect the response of chironomid communities to contaminants in receiving waters. The harsh conditions of highly eutrophic systems may accentuate the effects of even small amounts of toxic materials out of proportion to their actual concentration. Oligotrophic lakes may absorb the impact of greater amounts of toxic materials before their effects become obvious. In oligotrophic systems, however, toxic materials probably would remain biologically available for longer periods of time because the rates of sediment accumulation typically are lower in such systems. Eutrophic systems generally have higher rates of sediment accumulation, but the higher loading of organic materials and the probability of mobilization of toxic inputs in these sediments would offset any positive effects of burial that might accrue. In systems receiving high loadings of mineral sediments, however, mobilization probably would be reduced and the effects of burial of toxic materials more effective in the short term at least. The individual parameters governing receiving waters, therefore, must carefully be taken into account in assessing the response of any fauna to contaminant stress.

Application of Antennal Indices

Antennae, by virtue of their sensory function, may be the first to detect, and be affected by, toxic substances in the environment. In the experiments with DDE, the highest incidence of deformed antennae was found at the lowest concentrations of DDE. As the concentration of DDE increased, however, the incidence of deformities decreased. The inverse relationship between concentration and deformities suggests that larval antennae are more sensitive to low levels of DDE and thus should be more sensitive to low-level, chronic contamination. The decline in the incidence of deformed antennae as the concentration of DDE increased suggests that (1) the capacity of the antennae to respond at higher levels of contamination may become saturated or (2) the effects of higher concentrations are increasingly lethal towards the weaker members of the community, while those that do survive are more resistant to increasing stress. In the larvae exposed to DDE, the shift towards deformation in the more heavily sclerotized structures of the head capsule (W. F. Warwick, unpubl. data) suggests that antennae are unable to respond at higher concentrations of contaminants. However, the inverse relationship between concentration and ISAD seems to support the second scenario; although fewer deformed antennae occurred in larvae exposed to the higher concentrations of DDE, among those antennae that were deformed were individual antennae that were more severely deformed than those occurring in the lower concentrations. The

TABLE 11. Compounds tentatively identified from Tobin Lake Sites VII and VIII (adapted from Samoiloff et al. 1983).

Compounds tentatively identified in Site VII fraction 2 extracts

benzaldehyde	ethenylbenzene (styrene)	4-methylhexanal
1-hexyl-3-methylcyclopentane	diethyl phthalate	1-hexen-3-ol
tetradecanal	*o*-neoisomenthol	6-methyl-2-heptanone
1,9-nonanediol	6,10,14-trimethyl-2-pentadecanone	3,3-dimethylhexane
2-nonenal	citronellyl propionate	2,2,3,4-tetramethylpentane
cis-1,2-diethylcyclobutane	13-octadecenal	nonadecanol
2,3,3-trimethyl-1-butene	7-butylbicyclo[4.1.0]heptane	1,12-tridecadiene
2-methyl-3-pentanol	1-dotriacontanol	hexadecanol
trans-2-methylcyclopentanol	17-octadecenal	campesterol
2-hexanethiol	1,2-dimethylbenzene	3-methyl-2-cyclohexen-1-one

Compounds tentatively identified in Site VIII fraction 2 extracts

benzaldehyde	2-hexanethiol	5-octadecenal
1-hexyl-3-methylcyclopentane	1-ethoxy-2-heptanone	4-decanone
tetradecanal	dihydro-5,5-dimethyl-2(3H)-furanone	1,1,3-trimethylcyclopentane
1,9-nonanediol	2-ethyl-1-hexanol	isooctyl alcohol
2-nonenal	3,3-dimethylhexanal	cyclododecane
4-hydroxy-4-methyl-2-pentanone	2-methylbenzaldehyde	3,7-dimethyl-6-octen-1-ol
4-hydroxy-5-methyl-2-hexanone	3- or 4-methylbenzaldehyde	2-methyl-1-dodecanol
2-methyl-3-pentanol	3-propoxy-1-propene	cholesterol
1-phenylethanone	3,5-dimethylcyclohexen-1-one	γ-sitosterol

Compounds tentatively identified as occurring at both sites

benzaldehyde	1-hexyl-3-methylcyclopentane	tetradecanal
2-nonenal	1,9-nonanediol	

same relationship was apparent between the two Tobin Lake sites. The deformed antennae from Site VI, logically the more severely contaminated site, were on average less severely deformed than those at Site V; the most extreme examples of deformed antennae, however, were found at Site VI. In addition, the massively deformed larvae found at the site located farther downstream between Site VI and the Squaw Rapids dam were all second instar larvae (W. F. Warwick, unpubl. data). These data suggest that the effects of contaminants were being exerted at a very early stage of the larval cycle. The possibility that these larvae would attain mature larval status, let alone complete the life cycle, seems highly unlikely.

Comparison of deformed antennae from field and experimental samples suggests that the length of exposure to toxic substances is an important factor in the response to contaminants. In Tobin Lake where successive generations were exposed to contaminants in the reservoir, the basal segments of the antennae were often stunted, withered, and in some cases entirely lacking. In contrast, there was very little deformation apparent in the basal segments of antennae deformed by exposure to DDE. The only difference appears to be in the length of exposure time. Derr and Zabik (1972a, 1972b) presented evidence that showed a significant proportion (30–40%) of the burden of DDE in exposed adult females of *Chironomus tentans* was transferred to the next generation via the egg mass. Since the DDE experiments were single dose–response experiments, there was probably insufficient time for DDE to accumulate and affect the sturdier basal segments of the antennae. It would seem from this that the effects of contaminants are probably cumulative and the range of morphological response greater and more closely correlated with concentration gradients, the longer the exposure to chemical contaminants. The factor of exposure time and the effects of bioaccumulation and transmission of toxic substances from generation to generation should therefore be incorporated into future experimental designs and taken into account in comparisons between experimental and field data. These factors would also have very important implications for the genetic composition of populations under stress.

The examples from Tobin Lake suggest that some specificity of response to specific chemicals or classes of chemicals may be expected. Although much more work remains to be done before any definitive statement can be made about specificity, the presence of certain types of deformities, particularly the introduction of structures of unknown homology on deformed antennae, provides encouraging evidence. Some examples are the antennae bearing palmate-shaped and trident-shaped structures from Tobin Lake Sites V and VI. Fusing of antennal segments may also represent a specific response. According to ISAD, the response would be measured by the degree of fusing from the simplest case where two segments are fused to the most extreme case where all five segments are fused. The possibilities and benefits for specificity of response in the detection and assessment of contaminants in the environment are immense, but this potentially invaluable aspect of the method probably will not be easily come by.

Comparisons between the duplicate samples from the Tobin Lake and Last Mountain Lake sites indicate that results are generally reproducible between bulk samples from the same site. The data show, however, that very large samples probably will be required to get good statistical confidence limits where the incidence of deformed antennae is very low or where sampling locations are relatively closely spaced. The statistics may improve considerably, however, when the deformities found in the other structures of the larval body are included in the calculation of Total Morphological Response (TMR).

Priorities for Future Development

The indexing system, although presently based only on morphological deformities in the antennae of the genus *Chironomus*, has been set up in such a manner that deformities in other

morphological structures can be incorporated into the index under the column identified as "Other" in Table 6. Numerical totals based on realistic classifications for deformities in the mentum, ventromentum, mandibles, premandibles, epipharyngeal pectin, and other morphological structures can be added directly to the total for ISAD because the total number of larvae used is common in each case. The TMR would therefore be calculated as follows:

$$\mathrm{TMR} = \frac{\Sigma\,\mathrm{ISAD} + k\,\Sigma\,\mathrm{Other}}{n}.$$

At present, ISAD treats the genus *Chironomus* as a single unit. As the data base for the genus is expanded, it may be possible to further refine the index to larval type, and possibly to species. The response of different instars to contaminants may also provide some insight into the mechanisms by which deformities are induced.

The classification system must also be expanded to include genera other than *Chironomus*. Deformities have been reported in a number of different genera including *Protanypus*, *Stictochironomus*, *Procladius*, *Micropsectra*, *Tanytarsus*, and a few other unidentified Chironomini and Tanytarsini. Some of these genera have different numbers of segments in the antennae, but these will be easily accommodated in the open style of indexing.

Not all genera appear to react to the presence of contaminants in the same manner, however. Wiederholm (1984) reported that although 10% of the *Chironomus* larvae in Västeråsfjärden Bay were deformed, none of the more abundant larvae of *Microchironomus* present were deformed. The same author reported that 17.5% of the *Micropsectra* larvae in Åsfjorden Bay were deformed whereas none of the cohabitant *Heterotrissocladius* larvae were deformed. The deformities described by Wiederholm did not include deformed antennae, but his data serve to illustrate the point that not all genera respond to the presence of contaminants in the same way. Until sufficient comparable data become available, the number of larvae (n) used to calculate ISAD, and ultimately TMR, will of necessity be confined to those genera that do develop morphological deformities in the presence of contaminants. The bias introduced by this decision may in part be countered by including an estimate of the diversity of the accompanying benthic community in the overall interpretation of the state of that community's health.

The inclusion of other morphological structures and other genera in the classification index is particularly important from the point of view of palaeolimnological studies on the historical impact of contaminants. The fragile antennae very rarely are found attached to head capsule remains. Physical abrasion by the sedimentary matrix more often than not scours these sensitive structures away with the result that palaeo-interpretations must be confined to the surviving hard parts. Correlation between antennal deformities and deformities occurring in the hard structures of the head capsule, particularly the mentum, hopefully will be able to counter these deficiencies in the subfossil fauna. The current state of research precludes direct comparison between contemporary and subfossil fauna, but future research in all likelihood can reconcile these differences.

Acknowledgements

I gratefully acknowledge the continued assistance and inspiration provided by Dr. A. L. Hamilton and Max Dyniques. Drs. D. W. Schindler and E. D. Ongley and Mr. D. R. Kimmett critically reviewed the draft manuscripts. Ms. C. A. Casey provided major technical support throughout all aspects of the program. Illustrations were prepared by R. Bennet, T. Derrett, and N. Heaton. Dr. J. R. Vallentyne and P. Fee, J. P. Witteman, and R. D. Wickstrom contributed valuable suggestions. Funding was provided under the Toxic Chemicals Management Program and by the Inland Waters Directorate, Western and Northern Region.

References

Birkholz, D. A., M. R. Samoiloff, W. F. Warwick, G. R. B. Webster, and J. Witteman. 1980. The Tobin Lake Project. Project proposal and progress report, November 1980. Office of Industrial Research, University of Manitoba, Winnipeg, Man. 110 p. (Available from The Library, Freshwater Institute, 501 University Cres., Winnipeg, Man. R3T 2N6)

Brinkhurst, R. O., A. L. Hamilton, and H. B. Herrington. 1968. Components of the bottom fauna of the St. Lawrence Great Lakes. Great Lakes Institute, University of Toronto, Toronto, Ont. No. PR33. 50 p.

Chan, P. K., G. P. O'Hara, and A. W. Hayes. 1984. Principles and methods for acute and subchronic toxicity, p. 1–51. *In* A. W. Hayes [ed.] Principles and methods of toxicology. Student Edition. Raven Press, New York, NY.

Cook, W., and D. Veal. 1969. A preliminary biological survey of Port Hope Harbour. Report to the Ontario Water Resources Commission, Division of Laboratories, Biology Branch, p. 11. (Available from the Ontario Ministry of Environment Main Library, St. Clair Ave. W., Toronto, Ont. M4V 1P5)

Cushman, R. M. 1984. Chironomid deformities as indicators of pollution from a synthetic, coal-derived oil. Freshwater Biol. 14: 179–182.

Derr, S. K., and M. J. Zabik. 1972a. Biologically active compounds in the aquatic environment: the uptake and distribution of (1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene), DDE by *Chironomus tentans* Fabricius (Diptera: Chironomidae). Trans. Am. Fish. Soc. 101(2): 323–329.

1972b. Biologically active compounds in the aquatic environment: the effect of DDE on the egg viability of *Chironomus tentans*. Bull. Environ. Contam. Toxicol. 7(6): 366–368.

Dixon, W. J., and F. J. Massey. 1969. Introduction to statistical analysis. McGraw-Hill Book Co., London and New York. 638 p.

Hamilton, A. L., and O. A. Saether. 1971. The occurrence of characteristic deformities in the chironomid larvae of several Canadian lakes. Can. Entomol. 103(3): 363–368.

Hare, L., and J. C. H. Carter. 1976. The distribution of *Chironomus* (s.s.)? *cucini* (*salinarius* group) larvae (Diptera: Chironomidae) in Parry Sound, Georgian Bay, with particular reference to structural deformities. Can. J. Zool. 54: 2129–2134.

Koehn, T., and C. Frank. 1980. Effect of thermal pollution on the chironomid fauna in an urban channel, p. 187–194. *In* D. A. Murray [ed.] Chironomidae: ecology, systematics, cytology and physiology. Pergamon Press, Oxford and New York.

Lindeberg, and T. Wiederholm. 1979. Notes on the taxonomy of European species of *Chironomus* (Diptera: Chironomidae). Entomol. Scand. Suppl. 10: 99–116.

Mason, P. G., and D. M. Lehmkuhl. 1983. Effects of the Squaw Rapids hydroelectric development on Saskatchewan River Chironomidae (Diptera). Mem. Am. Entomol. Soc. 34: 187–210.

McIver, S. B. 1975. Structure of cuticular mechanoreceptors of arthropods. Annu. Rev. Entomol. 20: 381–397.

Petersen, L. B.-M., and R. C. Petersen. 1983. Anomalies in hydrophysiid capture nets from polluted streams. Freshwater Biol. 13: 185–191.

Saether, O. A. 1969. Some Nearctic Podonominae, Diamesinae, and Orthocladinae. Bull. Fish. Res. Board Can. 170: 154 p.

1970. A survey of the bottom fauna in lakes of the Okanagan Valley, British Columbia. Fish Res. Board Can. Tech. Rep. 196: 28 p.

1980. Glossary of chironomid morphology terminology (Diptera: Chironomidae). Entomol. Scand. Suppl. 14: 1–51.

Saether, O. A., and M. P. McLean. 1972. A survey of the bottom fauna in Wood, Kalamalka and Skaha lakes in the Okanagan Valley, British Columbia. Fish. Res. Board Can. Tech. Rep. 342: 27 p.

Samoiloff, M. R., J. Bell, D. A. Birkholz, G. R. B. Webster, E. G. Arnott, R. Pulak, and A. Madrid. 1983. Combined bioassay – chemical fractionation scheme for the determination and ranking of toxic chemicals in sediments. Environ. Sci. Technol. 17: 329–334.

Schlee, D. 1966. Präparation und Ermittlung von Messwerten an Chironomidae (Diptera). Gewässer. Abwasser. 41/42: 169–193.

Slifer, E. H. 1970. The structures of arthropod chemoreceptors. Annu. Rev. Entomol. 15: 121–142.

Slifer, E. H., and S. S. Sekhon. 1971. Circumfila and other sense organs on

the antennae of the sorghum midge (Diptera: Cecidomyiidae). J. Morphol. 133: 281–302.

SNODGRASS, R. E. 1935. Principles of insect morphology. McGraw-Hill Book Co., New York and London. 667 p.

SUBLETTE, J. E. 1979. Scanning electron microscopy as a tool in taxonomy and phylogeny of Chironomidae (Diptera). Entomol. Scand. Suppl. 10: 47–65.

SUBLETTE, J. E., AND M. F. SUBLETTE. 1974. A review of the genus *Chironomus* (Diptera, Chironomidae) VII. The morphology of *Chironomus stigmaterus* Say. Stud. Nat. Sci. 1(10): 1–65.

TENNESSEN, K. J., AND P. K. GOTTFRIED. 1983. Variation in structure of ligula of Tanypodinae larvae (Diptera: Chironomidae) Entomol. News 94(4): 109–116.

UTHE, J. F. [ED.] 1972. Mercury in the aquatic environment: a summary of research carried out by the Freshwater Institute, 1970–1971. Fish. Res. Board Can. MS Rep. Ser. No. 1167: 163 p.

WARWICK, W. F. 1980a. Palaeolimnology of the Bay of Quinte, Lake Ontario: 2800 years of culture influence. Can. Bull. Fish. Aquat. Sci. 206: 117 p.

1980b. Chironomidae (Diptera) responses to 2800 years of cultural influence; a palaeolimnological study with special reference to sedimentation, eutrophication and contamination processes. Can. Entomol. 112: 1193–1238.

1980c. Pasqua Lake, southeastern Saskatchewan: a preliminary assessment of trophic status and contamination based on the Chironomidae (Diptera), p. 255–267. *In* D. A. Murray [ed.] Chironomidae: ecology, systematics, cytology and physiology. Pergamon Press, Oxford, and New York.

1985. Morphological deformities in Chironomidae (Diptera) larvae as biological indicators of toxic stress. International Joint Commission Workshop on Persistent Toxic Substances and the Health of the Aquatic Community, 17–21 June 1985, Minneapolis, MN. (In press)

WARWICK, W. F., AND C. A. CASEY. 1982. Sampling chironomid communities in lakes. Can. Dep. Environ., Inl. Waters Dir., Natl. Water Res. Inst. Tech. Rep. W.N.R.-PR-82-02: 43 p.

WIEDERHOLM, T. 1984. Incidence of deformed chironomid larvae (Diptera: Chironomidae) in Swedish lakes. Hydrobiologia 109: 243–249.

ZACHARUK, R. Y. 1980. Ultrastructure and function of insect chemosensilla. Annu. Rev. Entomol. 25: 27–47.

ZACHARUK, R. Y., AND S. G. BLUE. 1971. Ultrastructure of the peg and hair sensilla on the antenna of larval *Aedes aegypti* (L.). J. Morphol. 135: 433–456.

Foraging Behavior of Hatchery-Produced Coho Salmon (*Oncorhynchus kisutch*) Smolts on Live Prey

Cynthia A. Paszkowski[1] and Bori L. Olla[2]

Oregon State University, College of Oceanography, Marine Science Center, Newport, OR 97365, USA

Paszkowski, C. A., and B. L. Olla. 1985. Foraging behavior of hatchery-produced coho salmon (*Oncorhynchus kisutch*) smolts on live prey. Can. J. Fish. Aquat. Sci. 42: 1915–1921.

We presented three groups of naive hatchery-produced, pellet-reared coho salmon (*Oncorhynchus kisutch*) with live, natural prey and observed their foraging behavior. Of 26 individuals examined, 20 captured one or more *Crangon* (sand shrimp) within 1 h of their first exposure to these prey. Six older (≥210 mm TL) accelerated smolts (underyearlings) displayed improved foraging performances with experience, capturing their first *Crangon* sooner during their second, compared with their first, exposure. Four of five of these fish successfully captured sand lance (*Ammodytes hexapterus*) within 1 h of their first presentation. Two groups of 10 recently smolted fingerlings (<190 mm TL), one accelerated and one conventionally reared (yearlings), were used in choice experiments and simultaneously offered equal masses of *Crangon* and pellets. Six of the accelerated and seven of the conventionally reared fish ate larger amounts of *Crangon* than pellets, with all 20 fish striking more at *Crangon* than at pellets. Our results suggest that a majority of hatchery-produced coho salmon smolts readily recognize, capture, and ingest natural prey and seem capable of foraging successfully upon release into the marine environment.

Nous avons présenté des proies naturelles à trois groupes de saumon coho (*Oncorhynchus kisutch*) produits en piscifacture et nourris de granulés et avons observé leur comportement dans la quête de nourriture. Des 26 individus observés, 20 ont capturé un *Crangon* (crevette grise) ou plus moins d'une heure après leur première rencontre avec ces proies. Six saumoneaux (alevins de moins d'un an) à croissance accélérée plus âgés (LT ≥ 210 mm) ont fait preuve de plus d'efficacité avec l'expérience, capturant leur premier *Crangon* plus tôt la deuxième fois que la première. Quatre de ces poissons sur cinq ont capturé des lançons (*Ammodytes hexapterus*) moins d'une heure après qu'ils eussent été mis en présence les uns des autres la première fois. Deux groupes de 10 tacons récemment devenus saumoneaux (LT < 190 mm), l'un à croissance accélérée et l'autre élevé de façon conventionnelle (poissons d'un an), ont été utilisés dans des expériences portant sur le choix de nourriture, où on leur a présenté en même temps des quantités égales de *Crangon* et de granulés. Six des poissons à croissance accélérée et sept de ceux élevés de façon conventionnelle ont consommé de plus grandes quantités de *Crangon* que de granulés, et les 20 poissons se sont attaqués davantage aux *Crangon* qu'aux granulés. Nos résultats donnent à penser que la plus grande partie des jeunes saumons cohos produits en piscifacture peuvent facilement reconnaître, capturer et ingérer des proies naturelles et qu'ils semblent capables de chercher leur nourriture avec succès une fois libérés dans le milieu marin.

Received January 25, 1985
Accepted August 16, 1985
(J8079)

Reçu le 25 janvier 1985
Accepté le 16 août 1985

Much of foraging theory rests on the supposition that animals can record and recall information about their environment and the other organisms inhabiting it and use this information, integrated with their own internal state, to make prudent decisions regarding how to forage, where to forage, and what to eat (Krebs 1978). On a short-term basis, recent experience allows adult animals to adjust their behavior so as to maximize foraging rewards and minimize the immediate risk of mortality. On a long-term basis, experience is intimately connected with the ontogeny of foraging patterns in developing individuals (e.g. Gray 1981). Changes in foraging behavior can sometimes reflect simple changes in dietary requirements or morphology with age, and the maturation of sensory or motor systems (e.g. Hunter 1972; Werner 1979). Changes can have more complex behavioral origins, such as exposure to novel food organisms and the subsequent development of search images, imprinting, or avoidance responses (Curio 1976).

The interaction between experience, foraging choices, and success is of particular importance to animals entering new environments. Such a situation confronts juvenile anadromous fishes as they trawl from freshwater to marine habitats and must adapt to a new array of food organisms, competitors, and predators. Even greater adjustments face hatchery-produced salmonids which have had no experience with capturing live prey and avoiding predators. Malnutrition has been proposed as one of the main sources of mortality for hatchery fish following release (Klak 1941; Reimers 1963; Gillen et al. 1981; Vinyard et al. 1982; Ersbak and Haase 1983).

We performed two series of experiments on coho salmon (*Oncorhynchus kisutch*) smolts to measure the capability of naive hatchery-produced, pellet-reared salmon to recognize and

[1]Present address: Department of Zoology, University of Alberta, Edmonton, Alta. T6G 2E9.

[2]Present address: Cooperative Institute for Marine Resources Studies, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, Hatfield Marine Science Center, Newport, OR 97365, USA.

capture live prey. The first set of experiments involved relatively large (≥210 mm total length TL) accelerated (underyearlings) smolts that had been held in seawater for more than 6 mo. Specifically, we addressed whether or not the foraging performances of hatchery-produced fish feeding on live prey improve with experience and whether prey-capture performance differs between crustacean and fish prey.

In the second set of experiments, smaller fish (<190 mm TL) that had recently smolted (in seawater < 5 wk) were used; these were more comparable in size and developmental stage with fish at the time of hatchery release than in the first experiments. Trials were conducted as preference tests that addressed the question of whether naive pellet-reared salmon prefer familiar artificial food or unfamiliar natural prey and then compared the performance of two groups of recently smolted fingerlings, one consisting of conventionally reared yearlings and the other of accelerated underyearlings.

Materials and Methods

Roles of Experience and Prey Type

All experiments were conducted at the Mark O. Hatfield Marine Science Center of Oregon State University. Fish used in the first set of experiments, conducted between 29 January and 9 March 1984, were older smolts, members of cohorts released by Oregon Aqua Foods (OAF) between June and August 1983 into Yaquina Bay, Oregon. They originated from a mixture of Green River, Washington, stock with Siletz River (Rocky Creek Hatchery), Oregon, stock. These fish, hereafter referred to as "OAF-1 smolts" (276–210 mm TL, 190.3–82.6 g), had been held in filtered seawater in 739-L circular tanks and maintained on Oregon moist pellets until October 1983. At that time they were used in a study of social behavior, conducted in the same experimental system as the feeding trials (Paszkowski and Olla 1985). The system consisted of two rectangular 2039-L tanks (2.4 × 0.9 × 0.9 m) that had three opaque fiberglass sides and transparent glass fronts. The tanks received a continuous flow of filtered seawater from Yaquina Bay (11–13°C). They were subjected to a natural photoperiod, seasonally adjusted, with a daytime light intensity of 8.3 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ at 0.75 m depth.

The salmon were introduced singly into the observation tank 1–2 d prior to the first feeding trial and received no food during this period. The first trial consisted of a pulsed presentation of 20 live sand shrimp (*Crangon nigricauda* and *C. franciscorum*; Decapoda: Crangonidae). *Crangon* were 25–35 mm in length (mass = 0.22–0.40 g, $\bar{x}$ = 0.33 g, N = 15) and were collected from Yaquina Bay or from 1.7 km offshore. *Crangon* were chosen as the invertebrate prey, not only because they were hardy and readily available, but because they are known, at times, to form a major component of the diet of coho salmon smolts in Yaquina Bay (Myers 1980).

Crangon were introduced into the tank two at a time in 60 mL of water through a PVC feeding tube located along the center of the back wall. Prior to each introduction, 60 mL of water was poured down the tube. This surface disturbance served to orient the fish, insuring their exposure to prey stimuli and standardizing initial conditions across introductions. Occasionally, *Crangon* became stuck in the tube and were immediately flushed out with additional water. *Crangon* typically drifted slowly to the bottom, although some individuals swam rapidly downward or horizontally. Trials consisted of 10 such presentations at 5-min intervals over a period of 45 min. An observer, located behind a blind, recorded the behavior of the fish during the presentations and for an additional 75 min; thus, each trial lasted 2 h (except for the first fish tested which was only observed for a total of 1 h). *Crangon* remained in the observation tank with the fish for 24 h, at which time surviving individuals were siphoned out.

Forty-eight hours after beginning the 20-*Crangon* trial (24 h after *Crangon* removal), we presented the same fish with 50 *Crangon*. We again poured 60 mL of water down the feeding tube to orient the fish and then added 50 *Crangon* simultaneously. We recorded the fish's behavior for 4–6 h, but analyzed only the first 2 h because most of the observed captures occurred in this period. After 24 h all remaining *Crangon* were removed. The same fish was presented 50 more *Crangon*, following identical procedures, 48 h after the beginning of the first 50-*Crangon* trial. After 24 h the *Crangon* and fish were removed from the observation tank and the fish was placed in an identical holding tank. This tank also contained the five other experimental salmon which were maintained there between trials and fed Oregon moist pellets on alternate days.

Between 11 and 15 March, after all six OAF-1 smolts had been tested on *Crangon*, we presented five of these fish (one salmon had died in the intervening period) with live vertebrate prey, sand lance (*Ammodytes hexapterus*), which were obtained from Washington. This species is also known to form part of the natural diet of coho smolts in Yaquina Bay and elsewhere along the Oregon coast (Myers 1980; Peterson et al. 1982).

Fish were placed in the observation tank 24 h before the prey presentation and were deprived of food during this period. At the start of each trial, 60 mL of water was poured down the central feeding tube to orient the fish. Ten *Ammodytes* (85–94 mm TL, mass = 0.80–1.80 g, $\bar{x}$ = 1.19 g) were then introduced simultaneously and fish and prey observed for 2 h. After this period, all animals were removed from the tank.

Preference Experiments

We were interested in examining the feeding behavior of recently smolted salmon comparable in size and development with fish released by Oregon hatcheries. Between 11 and 26 May 1984 we tested 10 accelerated underyearling coho smolts (129–149 mm TL, 15.9–26.5 g) from OAF. These fish, hereafter referred to as "OAF-2 smolts," belonged to a cohort released into Yaquina Bay in mid-May. They had been in seawater a maximum of 22 d when used in the experiment. Prior to and following feeding trials, these fish were held in 739-L circular flow-through tanks and maintained on pelleted food.

Experimental fish were introduced into the previously described observation tanks about 1 h after feeding on pellets in their holding tanks. A fish was held for 48 h without additional food before being used in a feeding trial. During the first 24 h, the newly introduced fish shared the tank with a prior resident (usually the fish used in the previous feeding trial), and their agonistic interactions were recorded as part of another study (Paszkowski and Olla 1985). The prior resident was then removed and the new fish remained alone for 24 h. Food presentations were similar to those described for OAF-1 smolts, except fish were offered *Crangon* and Oregon moist pellets simultaneously. At the beginning of a presentation, 60 mL of water was poured down a central feeding tube. After 5–10 s, two *Crangon* (15–25 mm in length) and 12 pellets were introduced through two other feeding tubes, located along the back wall of the tank and separated by 40 cm. We used this food

combination because the mass of one *Crangon* ($\bar{x}$ = 0.11 g, range = 0.04–0.21 g, N = 25) was approximately equal to the mass of six pellets ($\bar{x}$ = 0.02 g each, range = 0.01–0.03 g, N = 10). We repeated these presentations at 5-min intervals, alternating the respective positions of the *Crangon* and pellet tubes, until we had offered the fish a total of 20 *Crangon* and 120 pellets in 10 pulses over a 45-min period. We then observed the fish for an additional 75 min, resulting in an observation period of 2 h. At the end of this time all remaining pellets and *Crangon* were removed from the tank. If the fish had fed on *Crangon*, a second individual was introduced and we collected data on social interactions (Paszkowski and Olla 1985). If the fish failed to feed on *Crangon*, we conducted a second trial 24 h later, after which a second fish was introduced regardless of the trial's outcome.

Feeding trials, following identical procedures, were conducted between 20 April and 26 May for 10 recently smolted Trask River fish. These smolts were conventionally reared yearlings (151–188 mm, 25.4–50.8 g) which originated from Tillamook Bay, Oregon, stock and belonged to a cohort released in mid-April into the Trask River, Oregon. They had been in seawater a maximum of 32 d when used in the experiments. To compensate for the greater mass of these fish, we used larger *Crangon* (25–35 mm, $\bar{x}$ mass = 0.33 g, range = 0.22–0.40 g, N = 15) and pellets ($\bar{x}$ mass = 0.05 g each, range = 0.02–0.09 g, N = 21).

Data Analysis

We recognized several behavior patterns in analyzing feeding trials: (1) chase — a fish followed a prey organism without physically contacting it; (2) strike — a fish chased and physically contacted a prey organism; (3) capture — a fish held a prey organism in its jaws; (4) ingestion — a fish successfully swallowed a captured prey organism. For pellets, strikes always ended in ingestion.

We used the patterns described above, in conjunction with the timing of these events and the number of prey or pellets consumed, to evaluate the foraging performances of individual fish. We expressed food consumption in terms of percent body weight to account for size differences among the experimental fish. Body weights were measured directly or derived from length–weight regressions. Data were analyzed with Wilcoxon rank sum and signed-ranks tests (Daniel 1978; McClave and Dietrich 1979). In all cases, $P \leq 0.05$ was termed significant.

Results

Roles of Experience and Prey Type

All six OAF-1 smolts ate *Crangon* within 45 min of their first exposure to these organisms. When presented 20 *Crangon*, the fish consumed 2–12 (median = 11, N = 6) within 2 h (Fig. 1). When presented 50 *Crangon*, they consumed 12–33 (median = 21.5, N = 12) within 2 h. There was no significant difference, either in numbers of *Crangon* eaten or percentages of body weights consumed, between the first and second 50-*Crangon* trials (Fig. 1; Wilcoxon signed-ranks tests). Fish readily captured *Crangon* (N = 253) from the water column (39.8%), bottom (30.8%), water surface (20.4%), and walls (9.0%).

Although trial format was somewhat different between the first exposure to *Crangon* (20-*Crangon* trials) and the second and third exposures (50-*Crangon* trials), we can compare some aspects of the fish's reactions to food organisms across these trials to determine the effects of experience on foraging performance. For example, time of first strike did not differ significantly between the first and second trials, in spite of differences in *Crangon* density (e.g. 2 versus 50 *Crangon* present). However, fish ingested the first *Crangon* significantly sooner in the second trial (Table 1; Wilcoxon signed-ranks tests). Time of first strike and time of first ingestion did not differ significantly between the two 50-*Crangon* trials (Table 1; Wilcoxon signed-ranks tests).

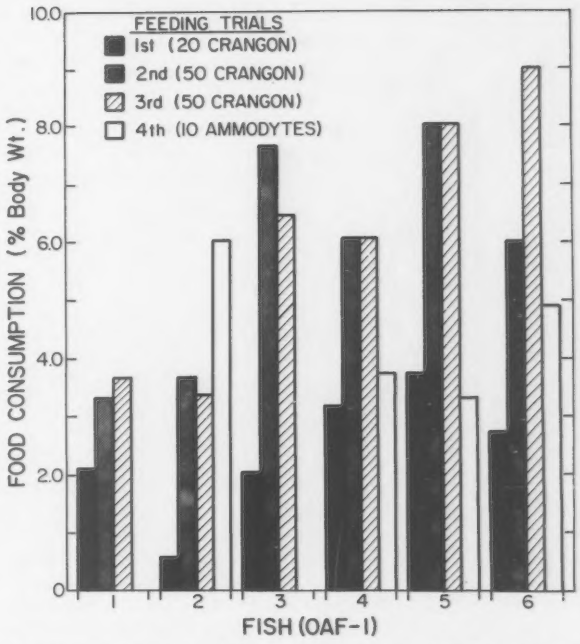


FIG. 1. Total amount of food eaten by six (1–6) OAF-1 smolts, expressed in terms of percent body weight, during 1-h (fish 1, 20-*Crangon* trial only) or 2-h exposures (all other trials) to 20 or 50 *Crangon* or 10 *Ammodytes* in four feeding trials.

Fish experienced with invertebrate prey did not greatly alter their foraging responses when presented vertebrate prey (Fig. 1). Four of the five fish tested ingested two to six *Ammodytes* and the fifth fish captured several but failed to ingest them. There was no significant difference between the time of first strike or the time of first ingestion between the first *Crangon* trial and the first *Ammodytes* trial (Table 1; Wilcoxon rank sum tests). Fish did engage in significantly more chases without contacting the prey when presented 10 *Ammodytes* (median = 88, N = 5) than when presented 20 *Crangon* (median = 9, N = 6; Wilcoxon rank sum test). While the total number of strikes did not differ significantly between *Crangon* and *Ammodytes* trials (median in both cases = 43; Wilcoxon rank sum test), this did represent more strikes per *Ammodytes* available and captured.

Preference Experiments

Five Trask River and nine OAF-2 smolts fed on *Crangon* within 2 h of their first exposure, and two additional Trask River fish fed during their second exposure. Smolts ingested one to six *Crangon* each (median for OAF-2 and Trask River = 2).

Fish of both stocks showed no preference for their accustomed food, pellets, over the novel food, *Crangon*. In both

TABLE 1. Time from trial initiation to first strike and first ingestion for OAF-1 smolts presented 20 and 50 *Crangon* and 10 *Ammodytes* for 2 h.

Individual fish	Trial 1 (20 *Crangon*)	Trial 2 (50 *Crangon*)	Trial 3 (50 *Crangon*)	Trial 4 (10 *Ammodytes*)
	Time to first strike (s)			
1	25	178	715	102
2	20	8	18	13
3	8	11	11	Not tested
4	9	8	9	16
5	910	13	8	8
6	164	9	7	74
	Time to first ingestion (s)			
1	905	204	1013	None eaten
2	29	8	210	71
3	2486	102	16	Not tested
4	311	12	9	129
5	1533	46	31	830
6	699	11	8	792

TABLE 2. Time from trial initiation until first strike at and ingestion of *Crangon* and first pellet ingestion for 10 OAF-2 smolts exposed to 20 *Crangon* and 120 pellets for 2 h. Total numbers of strikes directed at *Crangon* and pellets during this period are also presented. Fish 10 did not feed on *Crangon* during the first presentation and thus received a second presentation 24 h later, resulting in 4 h of exposure to food.

Individual fish	Time to first *Crangon* strike (s)	Time to first *Crangon* ingestion (s)	Time to first pellet ingestion (s)	Total no. of strikes	
				Crangon	Pellets
1	19	258	696	20	6
2	16	343	24	54	20
3	136	465	1359	29	4
4	159	159	None eaten	31	0
5	799	1237	325	64	38
6	79	628	919	34	1
7	102	626	15	35	8
8	39	58	314	81	32
9	54	59	399	62	15
10	117	None eaten	3402	26	9

TABLE 3. Time from trial initiation until first strike at and ingestion of *Crangon* and first pellet ingestion for 10 Trask River smolts exposed to 20 *Crangon* and 120 pellets for 2 h. Total numbers of strikes directed at *Crangon* and pellets during this period are also presented. Fish 2, 6, 8, 9, and 10 did not feed on *Crangon* during the first presentation and thus received a second presentation 24 h later, resulting in 4 h of exposure to food.

Individual fish	Time to first *Crangon* strike (s)	Time to first *Crangon* ingestion (s)	Time to first pellet ingestion (s)	Total no. of strikes	
				Crangon	Pellets
1	321	960	20	36	5
2	146	8 169	1 220	20	2
3	174	1 118	2 221	33	1
4	44	153	17	28	5
5	121	782	608	56	5
6	2411	11 450	13 617	4	0
7	321	3 010	16	80	7
8	1752	None eaten	None eaten	4	0
9	28	None eaten	None eaten	32	0
10	712	None eaten	None eaten	8	0

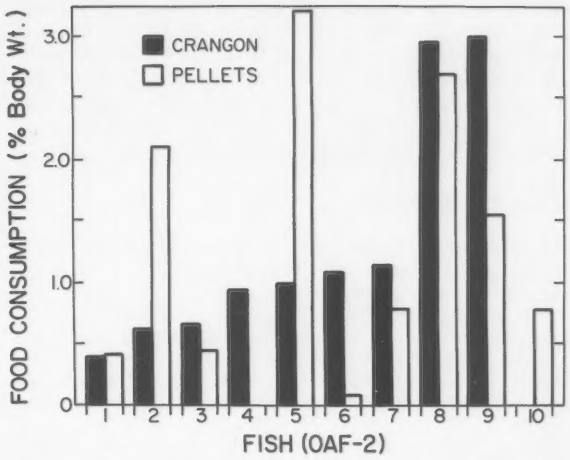


FIG. 2. Total amount of food eaten by 10 (1–10) OAF-2 smolts, expressed in terms of percent body weight, during their first 2-h exposure to 20 *Crangon* and 120 pellets. Fish 10 received a second 2-h exposure and consumed 1.65% of its body weight in pellets.

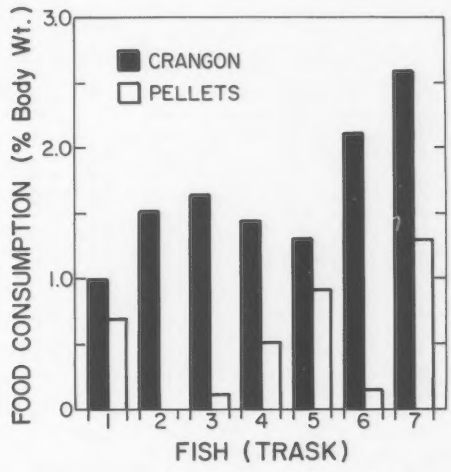


FIG. 3. Total amount of food eaten by seven (1–7) Trask River smolts during their first 2-h exposure to 20 *Crangon* and 120 pellets (fish 1, 3–5, 7) or the first exposure (second 2-h trial) in which they ate *Crangon* (fish 2, 6). Fish 2 ate 0.22% of its body weight in pellets during its first 2-h exposure to both foods, whereas fish 6 ate nothing. Three additional smolts (fish 8–10) ate no food during two 2-h exposures.

groups, there was no significant difference between the time the first pellet and the first *Crangon* were struck or ingested by fish which ate both foods (Tables 2, 3; Wilcoxon signed-ranks tests). Fish of both stocks invested more time and energy in taking *Crangon* than in taking pellets, since both groups directed significantly more strikes at *Crangon* than at pellets (Tables 2, 3; Wilcoxon signed-ranks tests). OAF-2 smolts invested a median number of 17.5 strikes for each *Crangon* ingested ($N = 9$) during their first exposure to live prey. The median number for Trask River smolts was 36 ($N = 5$).

Fifteen of the 17 recent smolts that fed ate both foods. However, six of nine OAF-2 fish and all seven Trask River fish ate greater proportions of their body weights in *Crangon* than in pellets during trials in which *Crangon* were eaten (Fig. 2, 3). This difference was significant for Trask River smolts, but not for OAF-2 smolts (Wilcoxon signed-ranks tests). Among fish that fed on *Crangon* there was no significant difference between the two stocks in the amount consumed, measured in terms of percent body weight (Fig. 2, 3; Wilcoxon rank sum test).

Discussion

A majority of the hatchery-produced, pellet-reared coho salmon smolts examined in our experiments had the ability to recognize and capture live invertebrate or vertebrate prey within hours of their first exposure to these food organisms (Fig. 1–3). This ability transcended genetic stock, rearing history, and time since smoltification. Our results suggest that coho salmon have an innate attraction to sensory stimuli characteristic of live prey (e.g. movement), and even naive fish are capable of appropriate predatory responses. The behavior patterns we observed for coho salmon feeding on *Crangon* and *Ammodytes* were similar to those described by Webb (1984) for rainbow trout (*Salmo gairdneri*) feeding on fathead minnows (*Pimephales promelas*). Both salmonid species used chases and steady swimming to capture prey.

Foraging performance improved with experience. Results for OAF-1 smolts (Table 1) suggest that one-trial learning was operating in which a single exposure produced a stable higher level of performance. This response was apparently generalized, extending from *Crangon* to a second, very different species, *Ammodytes*. Of course, the fish's foraging patterns differed somewhat between prey species. *Ammodytes* were more active and evasive than *Crangon* and captures required more chases and strikes.

The responses of OAF-1 smolts to live prey generally agreed with behavior patterns reported for "naive" fishes of other species presented novel foods under laboratory conditions. Most fishes readily attack new food types and soon adopt foraging tactics leading to higher success rates for searching and capturing (e.g. Ware 1971; Ringler 1979; Webb and Skadsen 1980; Gillen et al. 1981; Werner et al. 1981; Vinyard 1982). For example, Webb and Skadsen (1980) reported that 43% of naive, pellet-reared tiger muskellunge (*Esox lucius* × *E. masquinongy*) captured fathead minnows on their very first strike attempt; by the seventh attempt, success was 100%. Similarly, Gillen et al. (1981) found that the median time required by naive, pellet-reared tiger muskellunge to capture their first fathead minnow was 540 s. Hatchery-produced fish, previously exposed to live prey, required a median time of 75 s in the same experimental environment. We observed comparable patterns for coho salmon including immediate initial interest in live prey followed by improved foraging performances (Table 1). However, because we conducted a limited number of trials, we cannot definitely conclude that learning allowed OAF-1 smolts to attain some maximal level of foraging efficiency in the experimental environment (see Werner et al. 1981). Other laboratory studies, on a variety of teleost species, suggest that this process requires 5–8 d of exposure to experimental prey populations (e.g. Ware 1971; Godin 1978).

Recently smolted salmon, both underyearlings and yearlings, responded strongly to live prey in choice experiments. They displayed no consistent preference for artificial food (which was all they had previously eaten) when offered an alternative, natural food, *Crangon* (Fig. 2, 3). In fact, Trask River fish ate

significantly greater amounts of *Crangon* than pellets (Fig. 3). Trask River smolts invested more strikes per ingestion when capturing *Crangon* than did OAF-2 smolts. *Crangon* presented to Trask River smolts were proportionately larger (0.6–1.3% of fish's body weights) than *Crangon* presented to OAF-2 smolts (0.4–0.7% of body weights) which could have made successful handling more difficult.

Smolts of both stocks, even individuals that ingested no *Crangon*, invested more effort in attempts to capture live prey than pellets, as evidenced by the significantly larger number of strikes directed at *Crangon* (Tables 2, 3). Admittedly, the two types of food did not provide equivalent stimuli. Individual *Crangon*, with roughly six times the mass and five times the surface area of single pellets, presented larger targets, although all pellets combined presented a greater number of targets. Both food types were most obvious, and thus most available, as they drifted through the water column immediately following their introduction into the tank. Live and mobile *Crangon* repeatedly represented themselves by moving along the tank bottom or up into the water column. Pellets lying on the bottom, however, still attracted fish. In fact, 84.0% ($N = 149$) and 45.8% ($N = 26$) of pellets ingested by OAF-2 and Trask River smolts, respectively, were taken from the bottom.

Bryan (1973), in his extensive study of the role of experience on food choice in rainbow trout, found that fish previously fed only hatchery pellets switched from pellets and preferentially ate *Artemia* or *Tubifex* after single exposures to these live foods. In contrast, trout switched between different types of live food less rapidly and less completely. Bryan also noted that trout struck, but rejected, novel foods more frequently than familiar foods, much as coho salmon struck *Crangon* more frequently than pellets (Fig. 2, 3). In a finding paralleling our comparisons of OAF-2 and Trask River smolts, Bryan showed that naive hatchery-reared rainbow trout from three different stocks all preferred live prey (*Daphnia* and Ephemeroptera) over pellets. Comparing one domestic and two wild strains, he found no difference in the proportion of fish (less than 60%) that ate pellets when also offered live food. Likewise, hatchery-reared fish from all three strains readily distinguished food (pellets or plankton) from nonfood (sawdust or debris).

The results of our experiments and those of other investigators (e.g. Bryan 1973; Gillen et al. 1981) suggest that a majority of hatchery fish can switch with some facility from feeding on artificial food to capturing natural prey organisms, and actually prefer the latter food sources. We did observe, however, that a minority of hatchery-reared smolts (31.1%, $N = 45$) examined in this and related work (Paszkowski and Olla 1985) refused to eat any food, including pellets, in our experimental system. This suggests that a portion of the hatchery population may be very sensitive to environmental changes and has difficulty in adapting to new conditions in general. Gillen et al. (1981) found that 27% of hatchery-reared tiger muskellunge failed to adopt a diet of minnows after 14 d of exposure to live prey in ponds.

A behaviorally nonadaptable minority may contribute to the poor survival and feeding performance reported for hatchery-produced salmonids in natural environments (e.g. Reimers 1963; Ersbak and Haase 1983). However, the apparent ability of the majority of hatchery fish to readily recognize and capture live prey (e.g. Lord 1934) by no means insures long-term foraging success and survival following release. Mortality may be related to energetically inefficient foraging or social patterns (Fenderson et al. 1968; Moyle 1969; Bachman 1984), selection of suboptimal diets (Sosiak et al. 1979), competition from wild fish of the same or other species (Dickson and MacCrimmon 1982; Bachman 1984), or feeding behavior that increases predation risks (Vincent 1960; Moyle 1969). To determine which of these factors, if any, contribute to the success or failure of hatchery-raised coho salmon smolts in the marine environment requires more detailed study of foraging patterns and interactions with prey, predators, and competitors.

Acknowledgements

We thank J. Anderson, G. Braun, R. Brodeur, M. Davis, J. Grover, B. Mundy, and J. Shenker for helping to care for the fish and conduct the experiments. M. Davis also helped analyze data. Oregon Aqua Food, Inc. and Oregon Department of Fish and Wildlife supplied us with salmon. E. Clarke, B. Mundy, and J. Shenker supplied us with *Crangon*. Comments from N. Ringler improved this manuscript. The research was supported, in part, by the United States National Marine Fisheries Service (NOAA 84-ABC-00091) and Oregon Aqua Foods, Inc.

References

BACHMAN, R. A. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. Trans. Am. Fish. Soc. 113: 1–32.

BRYAN, J. E. 1973. Feeding history, parental stock, and food selection in rainbow trout. Behaviour 45: 123–153.

CURIO, E. 1976. The ethology of predation. Springer-Verlag, New York, NY. 250 p.

DANIEL, W. W. 1978. Applied nonparametric statistics. Houghton Mifflin Co., Boston, MA. 507 p.

DICKSON, T. A., AND H. R. MACCRIMMON. 1982. Influence of hatchery experience on growth and behavior of juvenile Atlantic salmon (*Salmo salar*) within allopatric and sympatric stream populations. Can. J. Fish. Aquat. Sci. 39: 1453–1458.

ERSBAK, K., AND B. L. HAASE. 1983. Nutritional deprivation after stocking as a possible mechanism leading to mortality in stream-stocked brook trout. N. Am. J. Fish. Manage. 3: 142–151.

FENDERSON, O. C., W. H. EVERHART, AND K. M. MUTH. 1968. Comparative agonistic and feeding behavior of hatchery-reared and wild salmon in aquaria. J. Fish. Res. Board Can. 25: 1–14.

GILLEN, A. L., R. A. STEIN, AND R. F. CARLINE. 1981. Predation by pellet-reared tiger muskellunge and minnows and bluegills in experimental systems. Trans. Am. Fish. Soc. 110: 197–209.

GODIN, J. J. 1978. Behavior of juvenile pink salmon (*Oncorhynchus gorbuscha* Walbaum) toward novel prey: influence of ontogeny and experience. Environ. Biol. Fishes 3: 261–266.

GRAY, L. 1981. Genetic and experimental differences affecting foraging behavior, p. 455–473. *In* A. C. Kamil and T. D. Sargent [ed.] Foraging behavior. Garland STPM Press, New York, NY.

HUNTER, J. R. 1972. Swimming and feeding behavior of larval anchovy *Engraulis mordax*. Fish. Bull. 70: 821–838.

KREBS, J. R. 1978. Optimal foraging: decision rules for predators, p. 23–63. *In* J. R. Krebs and N. B. Davies [ed.] Behavioural ecology. Sinauer Ass. Inc., Sunderland, MA.

KLAK, G. F. 1941. The condition of brook trout and rainbow trout from four eastern streams. Trans. Am. Fish. Soc. 70: 282–289.

LORD, R. F. 1934. Hatchery trout as foragers and game fish. Trans. Am. Fish. Soc. 64: 339–345.

MCCLAVE, J. T., AND F. H. DIETRICH II. 1979. Statistics. Dellen Publ. Co., San Francisco, CA. 681 p.

MOYLE, P. B. 1969. Comparative behavior of young brook trout of domestic and wild origin. Prog. Fish-Cult. 31: 51–59.

MYERS, K. W. W. 1980. An investigation of the utilization of four study areas in Yaquina Bay, Oregon by hatchery and wild juvenile salmonids. M.S. thesis, Oregon State University, Corvallis, OR. 234 p.

PASZKOWSKI, C. A., AND B. L. OLLA. 1985. Social interactions of coho salmon (*Oncorhynchus kisutch*) smolts in seawater. Can. J. Zool. 63: 2401–2407.

PETERSON, W. T., R. D. BRODEUR, AND W. G. PEARCY. 1982. Food habits of juvenile salmon in the Oregon coastal zone, June 1979. Fish. Bull. 80: 841–851.

REIMERS, N. 1963. Body condition, water temperature and over winter survival of hatchery-reared trout in Convict Creek, California. Trans. Am. Fish. Soc. 92: 39–46.

RINGLER, N. H. 1979. Selective predation by drift feeding brown trout (*Salmo trutta*). J. Fish. Res. Board Can. 36: 392–403.

SOSIAK, A. J., R. G. RANDALL, AND J. A. MCKENZIE. 1979. Feeding by hatchery-reared and wild Atlantic salmon (*Salmo salar*) parr in streams. J. Fish. Res. Board Can. 36: 1408–1412.

VINCENT, R. E. 1960. Some influences of domestication upon three stocks of brown trout (*Salvelinus fontinalis* Mitchell). Trans. Am. Fish. Soc. 89: 35–52.

VINYARD, G. L. 1982. Variable kinematics of Sacramento perch (*Archoplites interruptus*) capturing evasive and nonevasive prey. Can. J. Fish. Aquat. Sci. 39: 208–211.

VINYARD, G. L., R. W. DRENNER, AND D. A. HANZEL. 1982. Feeding success of hatchery reared kokanee salmon when presented with zooplankton prey. Prog. Fish-Cult. 44: 37–39.

WARE, D. M. 1971. Predation by rainbow-trout (*Salmo gairdneri*): the effect of experience. J. Fish. Res. Board Can. 28: 1847–1852.

WEBB, P. W. 1984. Body and fin form and strike tactics of four teleost predators attacking fathead minnow (*Pimephales promelas*) prey. Can. J. Fish. Aquat. Sci. 41: 157–165.

WEBB, P. W., AND J. M. SKADSEN. 1980. Strike tactics of *Esox*. Can. J. Zool. 58: 1462–1469.

WERNER, E. E. 1979. Niche partitioning by food size in fish communities, p. 311–322. *In* R. H. Stroud and H. Clepper [ed.] Predator–prey systems in fisheries management. Sport Fishing Institute, Washington, DC.

WERNER, E. E., G. G. MITTELBACH, AND D. J. HALL. 1981. The role of foraging profitability and experience in habitat use by the bluegill sunfish. Ecology 62: 116–125.

Effects of Successive Flow Perturbations on Stream Invertebrates

J. R. Irvine[1]

Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

Irvine, J. R. 1985. Effects of successive flow perturbations on stream invertebrates. Can. J. Fish. Aquat. Sci. 42: 1922–1927.

Initial stream flow changes after a period of constant flow have a much larger effect on invertebrate drift than subsequent changes. Constant discharge was maintained in two man-made streams for several months, after which discharge was kept constant on one stream (control) while in the other (treatment) it was increased fivefold and then returned to its original state three times between 17:00 and 21:00 in 1 d. This treatment was repeated on four successive days each week for 3 wk. On the first day of treatment the density of invertebrate drift increased in the treatment stream during the changes in discharge, the increase being less with each successive change. By the final day of treatment, however, the changes in discharge did not cause increases in drift, and this result is attributed to depletion of the benthos by repeated fluctuations in discharge. Surber sample estimates of benthic invertebrate density declined following flow changes. Many of the invertebrates displaced by flow changes were probably resident in sloughed off filamentous algae. I conclude that it is necessary to know the flow history of a river before being able to predict effects of flow perturbations.

Les premiers changements de débit d'un cours d'eau après une période de débit constant ont un effet beaucoup plus important sur la dérive des invertébrés que les changements ultérieurs. Le débit a été maintenu constant dans deux cours d'eau artificiels pendant plusieurs mois; par la suite, on a gardé le débit constant dans un cours d'eau (témoin) tandis que dans l'autre cours d'eau (subissant le traitement) on augmentait le débit à cinq fois sa valeur initiale pour le remettre comme il était trois fois par jour entre 17:00 et 21:00. On a répété ce traitement 4 jours consécutifs par semaine pendant 3 sem. Le premier jour du traitement, la densité d'invertébrés à la dérive a augmenté dans le cours d'eau subissant le traitement au cours des changements de débit, l'augmentation étant cependant moins importante avec chaque changement successif. Toutefois, le dernier jour du traitement, les changements de débit n'ont pas provoqué d'augmentation dans la dérive; ce résultat est attribué à l'appauvrissement du benthos en raison des fluctuations répétées du débit. Les estimations de la densité d'invertébrés benthiques faites à partir d'échantillons prélevés à l'aide de l'échantillonneur Surber ont diminué à la suite des changements de débit. Beaucoup des invertébrés déplacés par les changements de débit résidaient probablement dans les algues filamenteuses dépouillées. Je conclus qu'il est nécessaire de connaître le régime d'une rivière avant de pouvoir prédire les effets des perturbations de débit.

Received March 6, 1985
Accepted July 29, 1985
(J8135)

Reçu le 6 mars 1985
Accepté le 29 juillet 1985

Many workers have investigated the effects of flow changes on stream communities, often by testing for effects below dams (for recent reviews, see Baxter 1977; Ward and Stanford 1979; Brooker 1981; Milner et al. 1981; Stalnaker 1982; Lillehammer and Saltveit 1984). Unfortunately, it is often impossible for these studies to be conducted with scientific controls in space and time as emphasized by Green (1979). Flow assessment studies lacking control areas are not necessarily deficient if flow perturbations are abrupt and large. Flow change assessments, however, lacking proper temporal controls (Briggs 1948; Radford and Hartland-Rowe 1971; Spence and Hynes 1971; Kondratieff and Voshell 1981; Scullion et al. 1982) generally indicate a more serious deficiency, as often it is not known whether differences existed between the control area and the impacted area before the impact occurred. Man-made replicate stream channels (Ottaway and Clarke 1981; Corrarino and Brusven 1983) enable controlled tests on the effects of flow perturbations to be conducted with greater confidence that recorded changes are related to these perturbations.

Flow perturbations have been shown to cause both immediate and delayed (nocturnal) increases in invertebrate drift (Brooker and Hemsworth 1978; Corrarino and Brusven 1983; Scullion and Sinton 1983). The mechanisms for immediate increased drift associated with flood flows are clear, but this is not the case for smaller flow increments (Scullion and Sinton 1983), nor for delayed drift increases. Although most streams are affected by multiple changes in discharge, in many studies the effects of single flow changes have been considered. Information from the study of single flow changes is not sufficient to enable one to speculate on cumulative or long-term impacts of multiple flow disturbances. My objectives were to conduct a controlled experiment using man-made streams to document the effects of successive flow changes on invertebrates drifting as well as on

[1]Present address: Department of Fisheries and Oceans, Fisheries Research Branch, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6.

those living on the bottom, and to determine the mechanism by which initial flow increases caused drift densities to become greater.

Methods

The lower Waitaki River replicate stream channels (Irvine 1984) are approximately 32 km upstream from the mouth of the Waitaki River, South Island, New Zealand. Waitaki River water was discharged through a 30-ha irrigation headpond into a smaller, constant-level headpond, and then into five parallel man-made streams. Stream discharge was set according to a previously determined stage–discharge relationship using slide gates at the upstream end of each stream. The streams were approximately 100 m long and 3 m wide at their base with 2:1 side slopes and 1:900 hydraulic gradient. Each had four riffles approximately 19 m long alternating with three pools approximately 8 m long. Riffles and pools were numbered from the upstream end. Stream channels were excavated in 1980 and lined to a depth of 50 cm with rocks taken from a nearby side channel of the Waitaki River. Using the modified Wentworth particle size scale (Bovee and Cochnauer 1977), these rocks consisted of 10% gravel and 90% cobbles.

Water depths and velocities (at 0.6 of the depth from the water surface) were measured using an Ott current meter at 50-cm intervals along transects established at the midpoint of each riffle and pool. Water temperature was recorded not less than once a week using a maximum–minimum thermometer.

Both streams used in the experiment were maintained at a base flow of 75 L/s for several months before the experiment was begun. A fivefold flow change was chosen for the experiment because this was the maximum range over which fish traps, used in other experiments, could be maintained in the streams. Discharge in Stream 1, the control, was constant from 31 August 1982 until the experiment was completed, and discharge in Stream 2, the experimental stream, was constant from 15 November 1982 until 22 March 1983. Commencing on 22 March, for four consecutive evenings each week until 7 April, there were three daily fivefold increases and decreases in discharge in Stream 2. Each period of increased flow lasted 45 min with 45-min low flow periods between; flow increases and decreases occurred virtually instantaneously. The second period of increased flow commenced at sunset so that the first discharge fluctuation took place entirely during daylight and the third during darkness (Fig. 1).

Invertebrates

To determine whether fluctuating flows had any effect on the types and numbers of invertebrates living on the bottom, three Surber (Surber 1936) samples were collected from each of Riffles 1 and 4 in each stream on 21 March (preimpact) and 7 April (postimpact). The area sampled was 1/16 m^2 and the collecting net had a mesh size of 0.125 mm.

Duplicate invertebrate drift samples were taken from Riffle 4 of each stream during each sampling period (Table 1). To determine which organisms were entering the stream by drift from the ponds, one sample was also taken from the extreme upper end of Stream 1 during each period. The timing of drift samples was arranged to overlap with Stream 2 discharge fluctuations. Overnight and daytime samples were taken to assess delayed effects of fluctuating flows. Samples were taken 21–22 March to provide a control between the two streams, 22–23 March to assess initial impacts of fluctuating flows, and 7–8 April to determine whether varying flows had any impact after they had been varying for 3 wk.

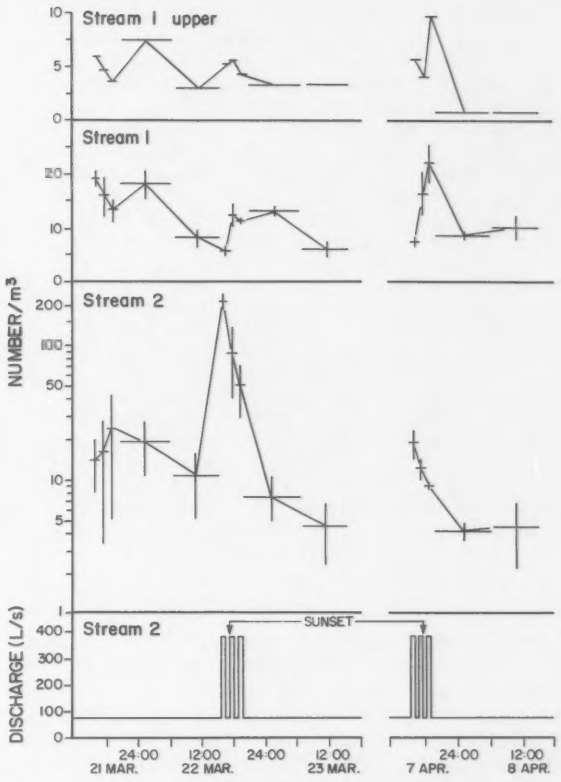


FIG. 1. Density of drifting invertebrates (excluding zooplankton and exuviae) in Streams 1 and 2 and discharge in Stream 2 (discharge in Stream 1 was always 75 L/s). Discharge in Stream 2 was also varied three times daily between 17:00 and 20:50 on each of 23, 24, 25, 29, 30, and 31 March and 1, 5, and 6 April but invertebrate drift was not sampled. Stream 1 upper was the sampling location at the upper end of Stream 1 (no replicate). Vertical bars indicate ranges between duplicate samples and horizontal bars indicate durations of sampling. Note logarithmic axis for Stream 2 drift.

Invertebrate drift samplers (Cushing 1964) had aperture openings of either 10 × 2 or 10 × 4 cm and 1-m-long collecting nets of 0.425-mm mesh size. Samples were taken with the upper edge of the frontal openings about 5 mm above the water surface to capture invertebrates, including those of terrestrial origin, drifting on or near the water surface as well as underneath. Water velocities were measured at the net mouth before and after each sampling period, and more frequently when flows were varying, using an Ott current meter. Sampling period durations were 75 min during flow changes and between 8 and 10.5 h for overnight and daytime samples (Table 1).

Drift densities (numbers per cubic metre) were calculated assuming 100% filtering efficiency of the drift samplers as was found by Cushing (1964).

The percentage of the benthos in the drift was estimated using the formula given by Elliott (1967):

$$P = \frac{xD \cdot 100}{X - xD}$$

TABLE 1. Timing of drift sampling in replicate Streams 1 and 2. Discharge in Stream 1 was always constant. Discharge was also varied three times daily between 17:00 and 20:50 on each of 23, 24, 25, 29, 30, and 31 March and 1, 5, and 6 April.

Date (1983)	Time of sampling	Stream 2 discharge pattern
21 March	17:00–18:15	Constant flow
21 March	18:30–19:45	Constant flow
21 March	20:00–21:15	Constant flow
21–22 March	21:30–07:30	Constant flow
22 March	08:00–16:30	Constant flow
22 March	17:00–18:15	Flow varied for the first time
22 March	18:30–19:45	Flow varied
22 March	20:00–21:15	Flow varied
22–23 March	21:30–07:30	Constant flow
23 March	08:00–16:30	Constant flow
7 April	16:35–17:50	Flow varied
7 April	18:05–19:20	Flow varied
7 April	19:35–20:50	Flow varied
7–8 April	21:05–07:30	Constant flow
8 April	08:00–16:00	Constant flow

where x = the drift density, D = the mean riffle depth (metres), and X = the mean benthos density (numbers per square metre) in Riffles 1 and 4. The 21 March benthic samples were assumed to be representative of the benthos during 21–23 March.

Large amounts of algae and other organic debris were sometimes retained by the drift samplers. After all invertebrates had been removed from these samples, the organic material remaining was dried at 100°C for 24 h and weighed.

To determine which invertebrates living amongst the attached algae could have contributed to the invertebrates retained by the drift samplers, three algae samples were taken from an adjacent replicate stream on 24 March. Care was taken to sample only the attached algae and not the substrate to which the algae were attached.

All invertebrates were initially preserved in 10% formalin and later transferred to 70% ethanol before processing. Samples were washed on a 0.25-mm sieve before counting. Invertebrates were identified at least to family and, where possible, to species.

Results

Water temperature gradually declined during the experiment from a maximum of 17.5°C the first week to a minimum of 11.0°C the final week. Water velocities and depths were similar in the two streams at base flow, but were significantly greater in Stream 2 at peak flow:

Characteristic	Habitat	Stream 1, 75 L/s	Stream 2	
			75 L/s	375 L/s
Velocity (cm/s)	Riffles	19.1	23.5	53.2
	Pools	6.6	4.4	17.5
	Overall	16.2	18.7	43.9
Depth (cm)	Riffles	11.2	8.7	17.4
	Pools	30.1	34.0	43.4
	Overall	16.2	15.5	24.9

Benthic Invertebrates

The flow fluctuations occurring in Stream 2 between 22 March and 7 April appeared to cause a decline in the density of benthic organisms. Average Stream 2 invertebrate densities decreased to a sixth during this period, while the density of invertebrates in Stream 1 decreased slightly in Riffle 1 but almost doubled in Riffle 4 (Table 2). As the variances of the samples were greater than the mean, total numbers of organisms per square metre were logarithmically transformed (Elliott 1973) which made the variances homogeneous, and a three-way streams-by-dates (pre- and post-impact)-by-riffles (Riffles 1 and 4) fixed effects analysis of variance was performed. According to Green (1979), the streams-by-dates interaction can be used to test the null hypothesis that there was no change in the density of benthic invertebrates in Stream 2 (impacted) that did not also occur in Stream 1 (control). Since this interaction was significant ($F = 22.98$; $P < 0.001$), fluctuating flows had a significant effect on the density of benthic invertebrates. However, Hurlbert (1984) stated that an areas-by-times interaction can be interpreted as an impact effect only if differences between locations remain constant over time, if there is no environmental impact, or the impact has no effect, and he felt this was not reasonable. In the analysis of variance, effects due to different streams, dates, and riffles were significant. The F values (probabilities in parentheses) for stream, date, and riffle effects were 6.42 (<0.05), 11.92 (<0.005), and 10.24 (<0.01), respectively.

Changes to the density and percentage occurrence of major taxonomic groups in the benthos of Stream 2, following the period of flow fluctuations, that did not also occur in the control stream are attributed to fluctuating flows. These changes were a decline in the density of all major forms except molluscs, a decrease in the proportion of oligochaetes, and an increase in the proportion of Elmidae larvae and chironomid larvae (Table 3).

TABLE 2. Mean number of benthic invertebrates (excluding terrestrial insects and zooplankton) captured per 100 cm^2 (standard error of the mean in parentheses) in the replicate streams.

Date (1983)	Stream 1		Stream 2	
	Riffle 1	Riffle 4	Riffle 1	Riffle 4
21 March	52.5 (26.3)	127.3 (99.9)	272.5 (205.1)	486.7 (171.8)
7 April	43.0 (10.8)	224.5 (95.3)	75.9 (25.0)	50.8 (21.0)

TABLE 3. Mean number per 100 cm^2 of eight taxonomic groups in benthic samples from the replicate streams. In parentheses, the frequency of each taxonomic group is expressed as a percentage of the total number of organisms. Data from Riffles 1 and 4 have been combined.

Stream	Date (1983)	Trichoptera larvae[a]	Mollusca[b]	Oligochaeta	Elmidae larvae	Chironomidae larvae	Arachnida[c]	Amphipoda[d]	Other
1	21 March	9.9 (11.04)	7.0 (7.77)	36.5 (40.58)	6.5 (7.18)	15.8 (17.62)	9.2 (10.26)	2.5 (2.82)	2.5 (2.73)
1	7 April	16.4 (12.28)	5.1 (3.81)	62.4 (46.65)	5.7 (4.29)	11.3 (8.43)	18.1 (13.54)	8.8 (6.56)	6.0 (4.45)
2	21 March	32.0 (8.42)	2.1 (0.54)	176.0 (46.36)	13.3 (3.49)	99.0 (26.08)	31.3 (8.25)	21.5 (5.66)	4.6 (1.22)
2	7 April	7.0 (11.12)	2.7 (4.25)	10.6 (16.67)	5.3 (8.42)	17.6 (27.74)	6.8 (10.74)	10.7 (16.84)	2.7 (4.21)

[a]Mainly *Oxyethira albiceps*.
[b]Mainly *Potamopyrgus antipodarum*.
[c]Mainly Hydracarina.
[d]Mainly *Paracalliope fluviatilis*.

TABLE 4. Mean number per cubic metre of eight taxonomic groups in drift samples from the replicate streams. In parentheses, the frequency of each taxonomic group is expressed as a percentage of the total number of organisms.

Stream	Date (1983)	Time	Trichoptera larvae[a]	Mollusca	Oligochaeta	Chironomidae larvae	Arachnida[b]	Amphipoda[c]	Terrestrial insects	Other
1	21 March	17:00–21:15	7.7 (47.83)	2.7 (16.77)	2.0 (12.42)	0.9 (5.59)	0.5 (3.11)	0.4 (2.48)	0.9 (5.59)	1.0 (6.21)
1	22 March	17:00–21:15	5.0 (51.02)	0.8 (8.16)	1.0 (10.20)	0.5 (5.10)	0.5 (5.10)	0.3 (3.06)	1.2 (12.24)	0.5 (5.10)
1	7 April	16:35–20:50	4.8 (31.58)	5.0 (32.89)	3.3 (21.71)	0.1 (0.66)	0.4 (2.63)	0.5 (3.29)	0.6 (3.95)	0.5 (3.29)
2	21 March	17:00–21:15	10.0 (55.87)	1.1 (6.15)	1.1 (6.15)	1.1 (6.15)	0.7 (3.91)	2.2 (12.29)	0.8 (4.47)	0.9 (5.03)
2	22 March	17:00–21:15	16.0 (13.69)	10.9 (9.32)	37.7 (32.25)	5.8 (4.96)	3.4 (2.91)	3.6 (3.08)	26.2 (22.41)	13.3 (11.38)
2	7 April	16:35–20:50	3.0 (22.22)	2.5 (18.52)	1.3 (9.63)	0.4 (2.96)	1.1 (8.15)	0.9 (6.67)	3.4 (25.19)	0.9 (6.67)

[a]Mainly *Oxyethira albiceps*.
[b]Mainly Hydracarina.
[c]Mainly *Paracalliope fluviatilis*.

Drifting Invertebrates

As the density of drifting organisms at the upstream end of Stream 1 ($\bar{x} = 4.5/m^3$) was much lower than the density in Riffle 4 ($\bar{x} = 12.5/m^3$) (Fig. 1), I concluded that most of the invertebrates drifting in Riffle 4 originated in the streams rather than upstream of them. In Stream 1 there were no consistent diel patterns in drift, nor were there large differences between days.

The three discharge fluctuations in Stream 2 on 22 March clearly increased the density of drifting organisms, but the increase was less with each successive fluctuation (Fig. 1). The three fluctuations on 7 April did not have a significant effect on invertebrate drift densities. There was no evidence for a delayed increase in invertebrate drift densities. In fact, in Stream 2 the lowest densities of drifting invertebrates occurred in the overnight and all day samples of 23 March and 8 April, so it is possible that these densities were reduced by varying flows.

Differences in drift in Stream 2 between the control day (21–22 March) and each of the days that flows were varied (22 March and 7 April) that did not also occur in Stream 1 over the same periods were assumed to be consequences of fluctuating flows. The three fluctuations occurring in Stream 2 on 22 March increased the density of all major drifting taxa (Table 4). The composition of the drift fauna was changed, with the percentage of the total drifting organisms made up by oligochaetes, molluscs, and terrestrial insects increasing and the percentage made up by trichopteran larvae, amphipods, and arachnids

TABLE 5. Percentage of the bottom fauna that was drifting in the water column over a unit area of stream bottom in the replicate streams.

Date (1983)	Stream 1 (control)	Stream 2 (treatment)
21–22 March (preimpact)	0.019	0.004
22–23 March	0.012	0.027
7–8 April	0.011	0.022

TABLE 6. Correlation coefficients (probabilities in parentheses) between total numbers of animals and dry weights of noninvertebrate organic matter in drift samples taken 21–23 March and 7–8 April 1983 in the replicate streams. Stream 1 upper was the sampling location at the upper end of Stream 1.

Stream	Trichoptera larvae[a]	Mollusca	Oligochaeta	Chironomidae larvae	Terrestrial insects	Total
1 upper	0.34 (>0.05)	0.70 (<0.01)	0.34 (>0.05)	0.65 (<0.01)	0.26 (>0.05)	0.70 (<0.01)
1	−0.06 (>0.05)	0.21 (>0.05)	0.06 (>0.05)	0.10 (>0.05)	0.33 (>0.05)	0.21 (>0.05)
2	0.74 (<0.01)	0.51 (<0.01)	0.97 (<0.01)	0.93 (<0.01)	0.49 (<0.01)	0.92 (<0.01)

[a]Mainly *Oxyethira albiceps*.

decreasing. The flow fluctuations on 7 April had far less impact on drifting invertebrates than those on 22 March. On 7 April, discharge fluctuations in Stream 2 appeared to decrease the density of drifting amphipods and increase the density of terrestrial insects. Fluctuating flows on 7 April also had little effect on the composition of the drift but may have increased the percentage occurrence of terrestrial insects and arachnids and decreased amphipods.

The percentage of the benthos in the drift did not vary markedly between dates in the control stream. In Stream 2, the percentage in the drift during both days of flow changes was higher than at other times (Table 5).

Drifting Organic Matter and Invertebrates in Algae

Dry weights of noninvertebrate drifting organic matter were highest during the flow changes in Stream 2 on 22 March, and were lowest at the top of Stream 1. In Stream 2 and for some taxa at the upper end of Stream 1, the amount of drifting organic matter exhibited similar patterns to the total numbers of drifting invertebrates, but it did not in Riffle 4 of Stream 1 (Table 6).

One attached algae sample was composed primarily of *Spirogyra* spp. and two were mainly *Cladophora* spp. Although there were differences in the order of abundance of major invertebrate taxa in the three samples, trichopteran larvae (primarily *Oxyethira albiceps*), oligochaetes, and chironomid larvae constituted the majority of organisms in each sample.

Discussion

Invertebrate drift density increased significantly during the first flow variation after several months of stable flows. Later that day, second and third flow fluctuations also increased the density of drifting invertebrates, but increases were less than that caused by the first fluctuation. At the end of 3 wk of fluctuating flows, fluctuations had no effect on the density of drifting invertebrates except for terrestrial insects.

Many of the invertebrates displaced by flow fluctuations probably had been resident in sloughed off filamentous algae. The three taxa common in attached algae (oligochaetes, chironomids, and trichopteran larvae) were also numerous in the drift. Dry weights of noninvertebrate organic matter (mostly algae) retained in the drift samplers were significantly correlated with total numbers of drifting invertebrates in Stream 2 with changing flows, but not at the bottom of the constant-flow stream. In Stream 2, correlations were highest for those three taxa most frequent in attached algae. O'Hop and Wallace (1983) and Irvine and Henriques (1984) also found a significant correlation between numbers of organisms and amount of organic material drifting and suggested that as more organic seston is transported during flow increases, animals associated with this debris are carried downstream. McLay (1968) suggested that while many invertebrates are able to take evasive action during a storm, oligochaetes and chironomids, which he found in high concentrations in algae on the substrate, cannot take such action. Mundie and Mounce (1976), Brooker and Hemsworth (1978), Matter et al. (1983), and Scullion and Sinton (1983) reported that densities of drifting chironomids and sometimes oligochaetes were high below dams during initial surges of water, but declined thereafter. Downstream displacement of riverine algae in response to fluctuating discharge may offer a suitable explanation for some of these findings.

Not all invertebrates displaced by peak discharges were associated with attached algae. For example, as arachnids were uncommon in attached algae and control flow drift samples, but increased in the drift during the first series of flow fluctuations, they were presumably unable to withstand increased water velocity during flow surges. Terrestrial insect drift densities increased during all flow changes; they were probably carried downstream as the stream rose.

Mundie and Mounce (1976) and Brooker (1981) suggested that flow changes may benefit downstream fish populations by increasing the number of drifting prey organisms. The most frequently consumed prey of underyearling brown trout (*Salmo trutta*) in the replicate streams were molluscs, amphipods, and trichopteran and chironomid larvae (Irvine 1984), and the drift density of each of these taxa increased during the first day of treatment. However, to be a practical management tool, successive flow perturbations would have to continue to increase invertebrate drift density, and results from my experiment indicate that this does not occur. Behavioural drift also appeared to be reduced following flow changes. Presumably invertebrate drift densities did not continue to increase because fewer invertebrates were available to drift. Decreased benthic densities were partly the result of loss of organisms to drift, but some taxa probably migrated downwards into the substrate to avoid scouring (Poole and Stewart 1976), thereby making them less easily captured by the Surber sampler.

My study illustrates the importance of knowing the flow history of a river before predicting effects of flow perturbations. The impact of flow changes will vary markedly depending on whether the system has recently been subjected to similar

perturbations. This means that, for regulated rivers, if time between flow changes is insufficient to allow for faunal recovery, there will be little additional effect on benthic invertebrates if the frequency of flow fluctuations is increased. Rates of recovery will vary considerably in different rivers. Gislason (1985) recommended that reducing the amplitude and duration of flow fluctuations will increase aquatic insect standing stocks. He found that the standing stock of benthic insects in the Skagit River was higher during a year of relatively stable flows than during the previous year when diel flow fluctuations occurred more frequently. Unfortunately, as Gislason (1985) did not have a control area, it cannot be said for certain whether density differences between the two years were the consequence of different flow patterns or some other reason. This demonstrates the importance of conducting controlled experiments to improve our understanding of the effects of flow changes on the aquatic community.

Acknowledgments

This research was financially supported by the Electricity Division (Ministry of Energy) and Fisheries Research Division (Ministry of Agriculture and Fisheries) of the New Zealand government. S. Lewthwaite assisted in the field and laboratory. I am grateful for the advice and encouragement given to me by J P. Graybill, S. F. Mitchell, and D. Scott.

References

BAXTER, R. M. 1977. Environmental effects of dams and impoundments. Annu. Rev. Ecol. Syst. 8: 255–283.

BOVEE, K. D., AND T. COCHNAUER. 1977. Development and evaluation of weighted criteria, probability-of-use curves for instream flow assessments: fisheries. Instream Flow Information Paper 3. U.S.D.I. Fish and Wildlife Service, Office of Biological Services. FWS/OBS-77/63. 49 p.

BRIGGS, J. C. 1948. The quantitative effects of a dam upon the bottom fauna of a small California stream. Trans. Am. Fish. Soc. 78: 70–81.

BROOKER, M. P. 1981. The impact of impoundments on the downstream fisheries and general ecology of rivers. Adv. Appl. Biol. 6: 91–152.

BROOKER, M. P., AND R. J. HEMSWORTH. 1978. The effect of the release of an artificial discharge of water on invertebrate drift in the R. Wye, Wales. Hydrobiologia 59: 155–163.

CORRARINO, C. A., AND M. A. BRUSVEN. 1983. The effects of reduced stream discharge on insect drift and stranding of near shore insects. Freshwater Invertebr. Biol. 2(2): 88–98.

CUSHING, C. E. 1964. An apparatus for sampling drifting organisms in streams. J. Wildl. Manage. 28(3): 592–594.

ELLIOTT, J. M. 1967. Invertebrate drift in a Dartmoor stream. Arch. Hydrobiol. 63(2): 202–237.

1973. Some methods for the statistical analysis of samples of benthic invertebrates. Scientific Publication No. 25, Freshwater Biological Association, Ferry House, U.K. 148 p.

GISLASON, J. C. 1985. Aquatic insect abundance in a regulated stream under fluctuating and stable diel flow patterns. N. Am. J. Fish. Manage. 5: 39–46.

GREEN, R. H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley & Sons, New York, NY. 269 p.

HURLBERT, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54(2): 187–211.

IRVINE, J. R. 1984. Effects of varying discharge on stream invertebrates and underyearling salmon and trout. Ph.D. thesis, University of Otago, Dunedin, New Zealand. 254 p.

IRVINE, J. R., AND P. R. HENRIQUES. 1984. A preliminary investigation on effects of fluctuating flows on invertebrates of the Hawea River, a large regulated river in New Zealand. N.Z. J. Mar. Freshwater Res. 18: 283–290.

KONDRATIEFF, B. C., AND J. R. VOSHELL, JR. 1981. Influence of a reservoir with surface release on the life history of the mayfly *Heterocloeon curiosum* (McDunnough) (Ephemeroptera: Baetidae). Can. J. Zool. 59: 305–314.

LILLEHAMMER, A., AND S. J. SALTVEIT [ED.] 1984. Regulated rivers. Universitetsforlaget AS, Oslo, Norway. 540 p.

MATTER, W. J., P. L. HUDSON, AND G. E. SAUL. 1983. Invertebrate drift and particulate organic material transport in the Savannah River below Lake Hartwell during a peak power generation cycle, p. 357–370. *In* T. D. Fontaine and S. M. Bartell [ed.] Dynamics of lotic ecosystems. Ann Arbor Science Publishers, Ann Arbor, MI.

MCLAY, C. L. 1968. A study of drift in the Kakanui River, New Zealand. Aust. J. Mar. Freshwater Res. 19: 139–149.

MILNER, N. J., J. SCULLION, P. A. CARLING, AND D. T. CRISP. 1981. The effects of discharge on sediment dynamics and consequent effects on invertebrates and salmonids in upland rivers. Adv. Appl. Biol. 6: 153–220.

MUNDIE, J. H., AND D. E. MOUNCE. 1976. Effects of changes in discharge in the Lower Campbell River on the transport of food organisms of juvenile salmon, Appendix A, 21 p. *In* R. Hamilton and J. W. Buell [ed.] Effects of modified hydrology on Campbell River salmonids. Environment Canada, Fish. Mar. Serv. Tech. Rep. Ser. No. PAC/T-76-20.

O'HOP, J., AND J. B. WALLACE. 1983. Invertebrate drift, discharge and sediment relations in a southern Appalachian headwater stream. Hydrobiologia 98: 71–84.

OTTAWAY, E. M., AND A. CLARKE. 1981. A preliminary investigation into the vulnerability of young trout (*Salmo trutta* L.) and Atlantic salmon (*S. salar* L.) to downstream displacement by high water velocities. J. Fish Biol. 19: 135–145.

POOLE, W. C., AND K. W. STEWART. 1976. The vertical distribution of macrobenthos within the substratum of the Brazos River, Texas. Hydrobiologia 50: 151–160.

RADFORD, D. S., AND R. HARTLAND-ROWE. 1971. A preliminary investigation of bottom fauna and invertebrate drift in an unregulated and a regulated stream in Alberta. J. Appl. Ecol. 8: 883–903.

SCULLION, J., C. A. PARISH, N. MORGAN, AND R. W. EDWARDS. 1982. Comparison of benthic macroinvertebrate fauna and substratum composition in riffles and pools in the impounded River Elan and the unregulated River Wye, mid-Wales. Freshwater Biol. 12: 579–595.

SCULLION, J., AND A. SINTON. 1983. Effects of artificial freshets on substratum composition, benthic invertebrate fauna and invertebrate drift in two impounded rivers in mid-Wales. Hydrobiologia 107: 261–269.

SPENCE, J. A., AND H. B. N. HYNES. 1971. Differences in benthos upstream and downstream of an impoundment. J. Fish. Res. Board Can. 28: 35–43.

STALNAKER, C. B. 1982. Effects on fisheries of abstractions and perturbations in streamflow, p. 366–383. *In* J. H. Grover [ed.] Allocation of fishery resources. Food and Agriculture Organization of the United Nations, Rome, Italy.

SURBER, E. W. 1936. Rainbow trout and bottom fauna production in one mile of stream. Trans. Am. Fish. Soc. 66: 193–202.

WARD, J. V., AND J. A. STANFORD [ED.] 1979. The ecology of regulated streams. Plenum Press, New York, NY. 398 p.

Influence of Mesh Composition, Velocity, and Run Time on the Catch and Length Composition of Juvenile Alewives (*Alosa pseudoharengus*) and Blueback Herring (*A. aestivalis*) Collected by Pushnet

B. M. Jessop

Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S. B3J 2S7

Jessop, B. M. 1985. Influence of mesh composition, velocity, and run time on the catch and length composition of juvenile alewives (*Alosa pseudoharengus*) and blueback herring (*A. aestivalis*) collected by pushnet. Can. J. Fish. Aquat. Sci. 42: 1928–1939.

Significant main and interaction effects were found in the influence of net mesh composition, net speed, and run time on the catch per cubic metre and length composition of juvenile alewives (*Alosa pseudoharengus*) and blueback herring (*A. aestivalis*) collected by a bow-mounted pushnet at dates when fish size and species abundance differed. Significant main effects for net type and net speed occurred for both catch and mean catch length for both species in August and, with one exception, for net speed in September. Interaction effects often differed between species but, for both species, the net type × net speed interaction was most common. Seasonal decreases in abundance and increases in the size of both species resulted in fewer and generally less significant main and interaction effects. Such interactions will complicate the comparison of studies where net type, net speed, and run time differ. I conclude that quantitative sampling of juvenile *Alosa* of 30–90 mm total length can be accomplished using a bow-mounted pushnet with mouth dimensions of 1.5 × 1.5 m, a uniform mesh size of 12.7 mm, speeds of 1.0–1.2 $m \cdot s^{-1}$, and run times of 5–8 min.

On a trouvé que le maillage, la vitesse et le temps de passage du filet avaient des effets majeurs et des effets d'interaction importants sur les prises par mètre cube et sur la répartition des longueurs de jeunes gaspareaux (*Alosa pseudoharengus*) et aloses d'été (*A. aestivalis*) capturés à l'aide d'un haveneau monté à l'avant à des dates où la taille des poissons et l'abondance des espèces différaient. Il y a eu des effets majeurs importants pour le type de filet et la vitesse du filet en ce qui concerne les prises et la longueur moyenne des prises pour les deux espèces en août et, à une exception près, pour la vitesse du filet en septembre. Les effets d'interaction ont souvent différé entre les espèces, mais, dans les deux cas, l'interaction la plus commune a été celle du type de filet par rapport à la vitesse du filet. Des diminutions saisonnières d'abondance et des augmentations de la taille des deux espèces ont entraîné un moins grand nombre d'effets majeurs et d'interaction qui, de façon générale, étaient moins importants. Ces interactions vont compliquer la comparaison d'études où le type de filet, la vitesse et le temps de passage du filet diffèrent. Je conclus que l'échantillonnage quantifatif de jeunes *Alosa* dont la longueur totale varie de 30 à 90 mm peut être effectué à l'aide d'un haveneau monté à l'avant ayant une ouverture de 1,5 × 1,5 m, un maillage uniforme de 12,7 mm, des vitesses de 1,0 à 1,2 $m \cdot s^{-1}$ et des temps de passage de 5 à 8 min.

Received March 21, 1985
Accepted July 29, 1985
(J8166)

Reçu le 21 mars 1985
Accepté le 29 juillet 1985

Estimates of stock abundance are essential to the assessment and management of marine and freshwater fisheries. In recent years, trawl surveys have proved useful sources of information on species abundance and distribution and may provide a reliable method of predicting future recruitment to fisheries (Doubleday and Rivard 1981). Trawl survey results are inherently variable, being influenced by factors such as the natural patchiness of population distributions, fish behavior, boat and gear types and operational methods, and survey design and environmental conditions (Byrne et al. 1981; Lenarz and Adams 1980; Leaman 1981). The variability and systematic measurement error (bias) associated with gear performance may be reduced by standardizing gear types and fishing protocol. Reductions, particularly if large, should increase the precision and accuracy of abundance estimates (Byrne et al. 1981; Carrothers 1981).

Evaluations exist of the effectiveness of various towed or pushed nets for the quantitative collection of plankton and larval and juvenile fish of several species (Lenarz 1972; Schnack 1974; Colton et al. 1980; Thayer et al. 1983; Gallagher and Conner 1983). For example, juvenile alewives (*Alosa pseudoharengus*) and American shad (*A. sapidissima*) are captured more efficiently during daylight by a bottom trawl, while juvenile blueback herring (*A. aestivalis*) and American shad are best caught at night by a bow-mounted pushnet (Kriete and Loesch 1980; Loesch et al. 1982). Few studies have evaluated

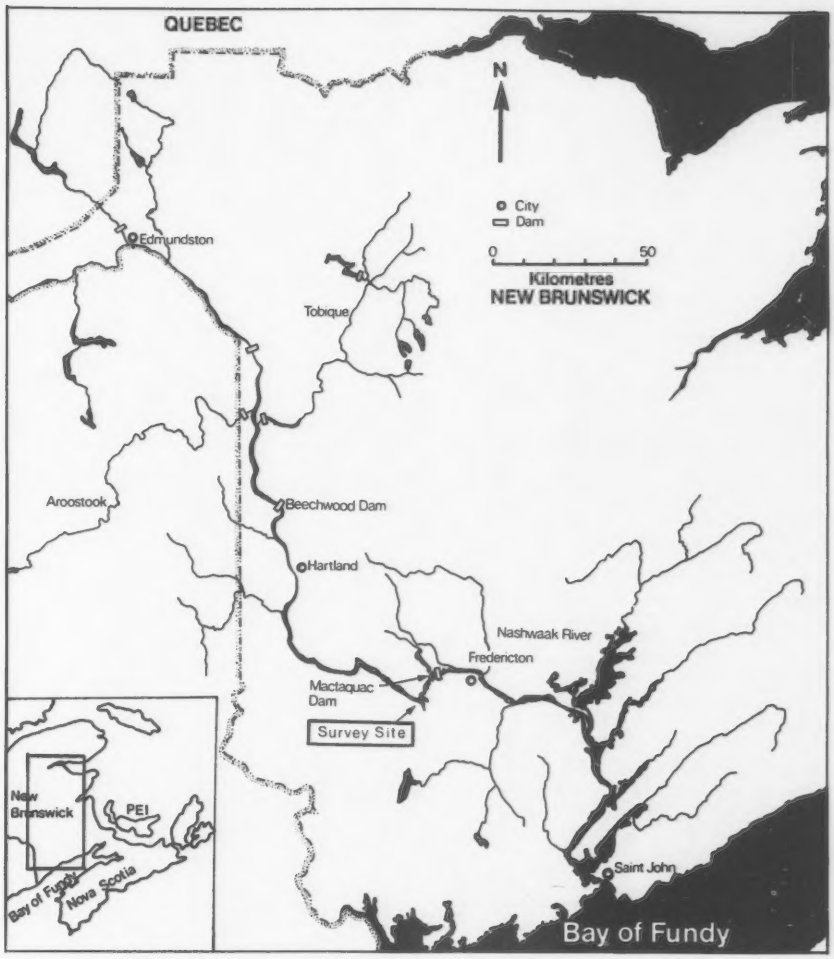


FIG. 1. Location of the net trial site on the Mactaquac Dam headpond, Saint John River, New Brunswick.

the effects of net speed and mesh size on catch abundance and length distribution.

My study was designed to evaluate the influence of differences in net velocity, run duration, and net mesh composition on the capture of juvenile alewife and blueback herring by a bow-mounted pushnet at dates when fish size and species abundance differed, and to derive recommendations for the efficient use of the pushnet to quantitatively sample juvenile alewives and blueback herring.

Materials and Methods

The pushnet frame, similar to that described by Kriete and Loesch (1980), was modified to adapt it to a 5.2-m fibreglass, cathedral-hulled boat powered by a single 0.75-kW outboard motor. Each specifically designed four-panel, tapered trawl was 4.6 m long with a 1.5 × 1.5 m mouth, the first net constructed entirely of 12.7-mm stretch mesh, size 210/8, knotless nylon and the second of 24.0-mm stretch mesh, size 210/15, knotless nylon in the 3.0-m body and 12.7-mm mesh in the 1.6 m cod. The estimated open area to mouth area ratio (R) was 5.8, and the filtration efficiency was over 95% for the 12.7-mm-mesh net based on the methods of Tranter and smith (1968).

The 2 × 3 × 5 factorial sampling design with three replications used two nets of different mesh sizes, three net speeds (0.6, 1.0, and 1.4 $m \cdot s^{-1}$), and five run times (1, 2, 4, 8, and 16 min). Each complete series of randomized speed–time sequences (45 pushes) for a given net formed a net trial. Two sampling periods, the first in early August (5, 6, and 9) and the second in mid-September (12 and 13), 1983, resulted in 4 net trials (2 nets × 2 periods), all in the same reach of the Saint John River, upstream of the Mactaquac Dam (Fig. 1). All were completed during full darkness on calm water in a single night except the 12.7-mm-mesh trial in early August which required two nights. The width of the reach (approximately 1 km) was divided into three rectangular zones. Runs were made sequentially from a start position randomly chosen from the four outer (shore) zone corners. Runs along the midline of the first zone followed the shoreline until one third of the push series had been completed. The boat was then moved to the middle of the next

TABLE 1. Mean catch of alewives and blueback herring, by trial period, net type, net speed, and run time, from the Mactaquac headpond, Saint John River, 1983 ($n = 3$ in all cases).

Speed ($m \cdot s^{-1}$)	Time (min)	August 5, 6, and 9				September 12 and 13			
		Alewife		Blueback herring		Alewife		Blueback herring	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
12.7-mm mesh									
0.6	1	8.0	1.7	17.3	14.0	0.3	0.6	0.3	0.6
	2	6.7	0.6	82.3	66.3	7.3	9.3	11.3	12.3
	4	9.0	6.6	18.0	5.2	0.7	1.2	2.3	2.3
	8	18.3	10.1	104.3	79.1	2.3	1.5	1.0	0.0
	16	35.0	34.6	41.0	28.2	5.0	4.0	3.7	5.5
1.0	1	7.0	7.9	76.3	58.2	3.3	3.2	0.0	0.0
	2	36.3	30.6	71.7	12.3	8.3	5.5	1.3	1.5
	4	26.7	15.3	213.7	179.3	29.0	13.0	2.0	1.0
	8	27.0	11.4	460.7	665.5	32.3	8.5	5.0	5.3
	16	131.0	22.3	2322.0	1100.4	90.3	48.6	161.7	247.4
1.4	1	15.7	10.1	70.7	100.8	7.3	4.0	1.0	0.0
	2	48.3	35.1	274.3	174.1	32.7	18.7	4.0	4.6
	4	61.0	33.1	591.0	631.5	16.3	4.6	2.7	2.1
	8	141.0	50.6	1276.7	535.5	94.3	52.4	2.7	3.1
	16	116.7	81.1	2119.0	986.7	158.7	70.9	5.7	3.8
24.0–12.7-mm mesh									
0.6	1	0.3	0.6	2.3	2.5	0.7	1.2	0.3	0.6
	2	1.7	2.1	14.3	9.1	1.0	1.0	0.0	0.0
	4	0.3	0.6	6.7	3.5	2.3	2.1	0.3	0.6
	8	0.3	0.6	11.0	12.8	5.0	4.0	1.7	1.2
	16	58.3	94.1	9.3	6.7	3.0	2.0	2.7	1.5
1.0	1	12.0	8.5	31.3	2.5	2.3	2.5	0.3	0.6
	2	10.7	7.5	7.7	8.1	7.3	6.7	1.0	1.0
	4	105.7	126.5	242.0	248.4	13.7	3.8	4.0	3.0
	8	73.3	73.7	161.7	120.9	44.0	27.8	10.3	3.8
	16	776.7	985.5	187.7	233.3	86.0	37.2	19.7	14.3
1.4	1	12.0	8.7	5.7	4.6	12.0	3.0	1.0	1.0
	2	13.7	10.8	36.7	54.8	32.7	23.0	2.3	2.1
	4	51.7	42.8	25.0	24.3	68.3	71.0	3.7	3.1
	8	111.3	85.6	301.7	351.5	196.0	208.7	1274.0	2191.9
	16	259.0	233.8	420.3	428.3	316.7	325.3	13.0	8.7

zone and the direction reversed. This procedure was repeated until the net trial was completed. During all runs the top of the net mouth was set about 15 cm beneath the water surface.

A calibrated flowmeter was used to determine the relationship between tachometer rpm and velocity for each net prior to the trials. Three 3-min runs were made for each net at each of six selected rpm settings. Over the range of velocities (0.4–1.5 $m \cdot s^{-1}$), the relationships were linear ($r^2 > 0.99$ in each case). Thus, for each net, use of the appropriate motor rpm resulted in the desired net velocity $\pm$ 0.05 $m \cdot s^{-1}$ in almost all cases. Discrepancies, all small, were attributed to operational difficulties in maintaining constant rpm and attaining the required rpm setting on start-up, particularly at lower speeds and shorter run times.

Catches were preserved in 4% formalin before being sorted and enumerated by species within 2 wk of capture. Alewives were distinguished from blueback herring on the basis of criteria in Lippson and Moran (1974). Numerous "positive" and all questionable identifications based on body conformation and size were confirmed by peritoneal color. The total length of all fish in samples of $\leq$40 fish of each species was measured to the nearest 1 mm. Subsamples of 40 fish were measured from larger samples.

Maximum body depth and depth at insertion of the pectoral fin were measured on juvenile fish collected during 1984. Preserved fish were in 2% formalin for a maximum of 6 h before measurement.

A fixed effects, 2 × 3 × 5 factorial analysis of variance (ANOVA) with three replications was employed to analyze the catch per cubic metre filtered of each species for each time period. The fixed model is, I believe, appropriate because the net speeds and run times were selected a priori. Net speeds were chosen to represent low, medium, and high speeds relative to those achievable by the equipment used and attainable by juvenile *Alosa* in the size range of interest. Run times were chosen to represent a practical range and to provide information on fish distribution patterns. Also, these factors can be considered, for practical purposes, as under control of the investigator and measured without error.

All analyses used the SPSS MANOVA program (Hull and Nie 1981). Catch per cubic metre (X) was transformed ($\log_e (X + 0.001)$) and was analyzed using the method of fitting constants

appropriate for a balanced, orthogonal model. The regression approach appropriate for an unbalanced, nonorthogonal model was used to analyze the length data, with the replicated three-way factorial cell means used rather than individual length measurements so as to be on the same sampling basis as the catch per unit volume analysis.

Tests were made of the assumptions of normality of distribution and homogeneity of variances underlying the ANOVA procedure. Main and interaction effect means were compared by the protected least significant difference (PSD) method.

Results

Filtration efficiencies were similar in that equal net speeds resulted in equal volumes of water filtered. Due to porosity differences, however, the drag of the 12.7-mm net was 1.14 times that of the 24.0-mm net, determined from differences in the motor rpm settings required to achieve a net speed of $1.0\,m\cdot s^{-1}$. As speeds increased, relatively more power was required to push the smaller mesh net, resulting in proportionately higher filtration pressures. The large open areas of each net reduced the likelihood of backwash and a pressure wave.

Within each experiment, replicate runs exhibited a high degree of variability in catch of both species for each net type, speed, and run time combination (Table 1). Mean catch was positively correlated with the variance and increased roughly linearly with run time. Logarithmic transformation of the catch produced homogeneous cell variances except for catches of blueback herring in September when the heterogeneity was attributed to two unusually large samples. Transformation also reduced the right skewness of the catch frequencies and made some symmetric.

A value of $K = 0.001$ in the relationship $\log_e(X + K)$ proved the most effective, of the values examined, in reducing the heterogeneity of cell standard deviations (and variances) and in normalizing the distribution of cell means. As the value of K was reduced, the positive relationship between the cell means and standard deviations was reduced or eliminated, Cochran's C (see Steel and Torrie 1980, p. 472) values were reduced in level of significance, and the distribution of means changed from right skewed to a more normal distribution. (Table 2). The ANOVA model also accounted for more variation (with one exception), as assessed by the coefficient of determination (r^2), as the value of K was reduced. The F-values became significant or highly significant five times as K was changed from 1.0 to 0.001 and the model fit was improved; nonsignificance occurred once. Although not optimum, a value of $K = 0.001$ satisfactorily met the requirements of a transformation for both species.

Length distributions of both species tended to be normal, if somewhat right skewed, for sample sizes >30 but platykurtic for alewives and leptokurtic for blueback herring. Sample unit mean lengths and cell mean lengths were essentially normally distributed for both species except for two outliers in the September trials of two unusually large (64 and 85 mm) blueback herring in samples of small size. Cell mean lengths plotted against their standard deviation and variance confirmed their independence within each experiment but also indicated some heterogeneity. Much of this heterogeneity, confirmed by Cochran's C test ($P > 0.001$ in all cases), can be attributed to relatively few unusually large or unusually small variances.

In early August, when alewife lengths averaged 55.1 mm and blueback herring averaged 37.9 mm, catches of both species were significantly influenced by both net type and net speed but not by run time (Table 3). The values of $\log_e(X + 0.001)$ mean catch per cubic metre (three replications) for each significant relationship were as follows:

TABLE 2. Relationship between ANOVA model fit and homogeneity of variances, as represented by the coefficient of determination (r^2) and Cochran's C and different values of K in the relationship $\log_e(X + K)$, for the analysis of juvenile alewife and blueback herring catch·m^{-3} from the Mactaquac headpond, Saint John River.

Net trial	Value of K	r^2	Cochran's C	P (df = 2, 30)
Aug. 5, 6, and 9				
Alewife	1.0	0.410	0.539	0.001
	0.25	0.437	0.388	0.001
	0.01	0.573	0.127	0.58
	0.001	0.682	0.134	0.46
Blueback herring	1.0	0.613	0.180	0.10
	0.25	0.645	0.145	0.32
	0.01	0.670	0.140	0.38
	0.001	0.658	0.124	0.64
Sept. 12 and 13				
Alewife	1.0	0.487	0.326	0.001
	0.25	0.522	0.274	0.003
	0.01	0.680	0.127	0.58
	0.001	0.691	0.160	0.19
Blueback herring	1.0	0.325	0.971	0.001
	0.25	0.330	0.920	0.001
	0.01	0.399	0.598	0.001
	0.001	0.449	0.299	0.001

TABLE 3. Factorial, fixed effects analysis of variance, by net type, velocity, and run time, of catch·m^{-3} for juvenile alewives and blueback herring collected by pushnet in the Mactaquac headpond, Saint John River, August 5, 6, and 9 (data transformed: $\log_e(X + 0.001)$).

		Alewife			Blueback herring		
Source	df	MS	F	P	MS	F	P
Net type (A)	1	12.1676	11.67	≪0.01	72.4449	49.33	≪0.01
Net speed (B)	2	30.4644	29.22	≪0.01	20.3834	13.88	≪0.01
Run time (C)	4	0.9436	0.91	0.47	0.2953	0.20	0.94
Replication	2	0.1355	0.13	0.88	2.2114	1.51	0.23
A × B	2	15.8080	15.16	≪0.01	2.2329	1.52	0.23
A × C	4	1.7373	1.67	0.17	0.7519	0.51	0.73
B × C	8	0.9308	0.89	0.53	4.5704	3.11	0.01
A × B × C	8	0.9138	0.88	0.54	1.7688	1.20	0.31
Error	58	1.0426			1.4687		
Total	89	2.1453			2.9459		

	Net type (mm mesh)		Net speed (m·s^{-1})		
	12.7	24.0–12.7	0.6	1.0	1.4
Alewife	−3.0414	−3.7776	−4.5712	−2.8736	−2.7837
Blueback	−1.4175	−3.2126	−3.2660	−1.8239	−1.8554

The 12.7-mm net captured more of both species than the 24.0-mm net. A significant increase in the catch of each species occurred between 0.6 and 1.0 m·s^{-1} but catches were similar at 1.0 and 1.4 m·s^{-1} (PSD = 0.53 and 0.63 for alewives and blueback herring, respectively; $P = 0.05$).

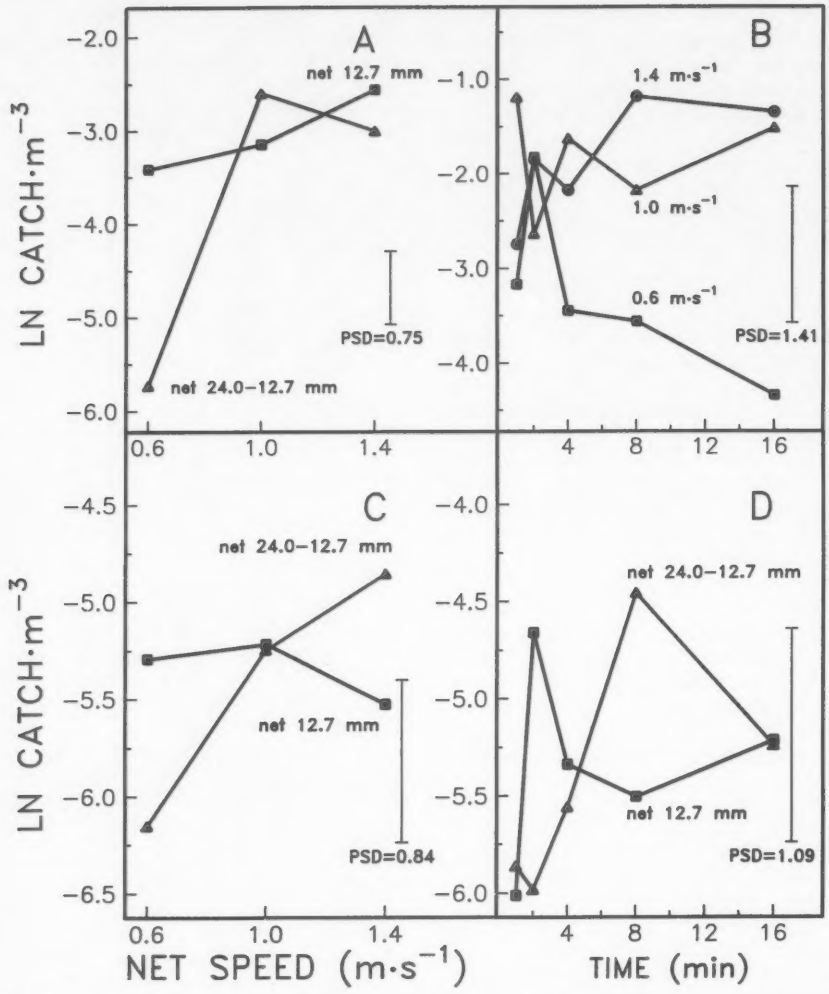


FIG 2. Relationship between (A) net speed and catch $\cdot m^{-3}$, by net type, for the capture of juvenile alewives in August, (B) net speed and catch $\cdot m^{-3}$, by run time, (C) net speed and catch $\cdot m^{-3}$, by net type, and (D) net speed and catch $\cdot m^{-3}$, by run time, for the capture of juvenile blueback herring in September using a pushnet in the Mactaquac Dam headpond, Saint John River. Catch $\cdot m^{-3}$ is $\log_e (X + 0.001)$ transformed.

Two significant first-order interactions occurred. For alewives, the effect of net speed on catch depended upon net type (Table 3; Fig. 2A). Higher speeds tended to produce higher catches for both nets, although the speed difference necessary to increase the catch rate was greater for the 12.7-mm net than for the 24.0-mm net. At speeds of 1.0 and 1.4 $m \cdot s^{-1}$ the catches were similar for both net types, but at 0.6 $m \cdot s^{-1}$ the 12.7-mm net caught more fish. For blueback herring, the net speed × time interaction produced an increasing catch with increasing run time at 1.4 $m \cdot s^{-1}$, no trend at 1.0 $m \cdot s^{-1}$, and a decline at 0.6 $m \cdot s^{-1}$ (PSD = 1.41; $P = 0.05$; Fig. 2B).

The effects of net type, velocity, and run time on the total length of fish captured differed between species (Table 4). For both species, main effects for net type and net speed, but not for run time, were significant. The unweighted (three replications) mean lengths (millimetres) for each significant treatment effect are compared below:

	Net type (mm mesh)		Net speed ($m \cdot s^{-1}$)		
	12.7	24.0–12.7	0.6	1.0	1.4
Alewife	52.7	55.1	51.7	51.9	57.3
Blueback	37.6	38.5	37.8	38.0	38.5

Fish of both species were shorter in catches by the 12.7-mm net than in catches by the 24.0-mm net. Fish captured at 1.4 $m \cdot s^{-1}$ were longer than those caught at either 1.0 or 0.6 $m \cdot s^{-1}$, at which speeds their lengths were similar (PSD = 2.36 for alewives and 0.59 for blueback herring; $P = 0.05$).

The significant main effects were subsumed by significant

first- and second-order interaction effects. Net type × net speed was significant for both species, net type × run time was significant for alewives, and net type × net speed × run time was significant for blueback herring. A high degree of between-sample-unit variability existed for blueback herring, but not for alewives.

Net type × net speed, the most significant of the two-way interactions, was similar for both species. It resulted in longer alewives and blueback herring being collected in the 12.7-mm net when net speed was increased from $0.6\,m\cdot s^{-1}$ but no difference in lengths at speeds of 1.0 and $1.4\,m\cdot s^{-1}$ (Fig. 3B, 3F). For the 24.0-mm net, the interaction was more complex and lengths of both species were shorter at $1.0\,m\cdot s^{-1}$ than at the other two speeds, at which lengths were similar. The high mean alewife length at $0.6\,m\cdot s^{-1}$ (56.2 mm) may be partly a consequence of the small sample size (about 0.15 that of the other treatment means) and the use of unweighted means. A larger sample size might have produced a smaller mean length; the trend to increasing mean length with increasing run speed would then apply to both nets. The same cannot be said for the blueback herring, which indicates that there may be substance to the complexity of interaction. The net type × run time interaction was weak ($P = 0.04$), and except for the 1-min run time, alewives captured by the two net types were similar in length at each run time (Fig. 3A).

For blueback herring, the moderately significant ($P = 0.03$), three-way interaction effect revealed no general pattern in lengths in relation to net speed and run time for different net types (Fig. 3D and 3E). The dynamics of fish capture, avoidance, and extrusion under these conditions appear complex, and sampling variability may exert a greater influence on the impact of these variables than with lower order interactions.

By mid-September, alewives had attained a mean length of 62.1 mm and blueback herring 43.9 mm, representing growth of 13 and 16%, respectively. Both species had declined in relative abundance, alewives by 42% and blueback herring by 83%.

Alewife catch per cubic metre increased significantly as net speed increased ($\log_e$ transformed treatment means: −5.4205, −3.7478, and −2.8852 at 0.6, 1.0, and $1.4\,m\cdot s^{-1}$; PSD = 0.47, $P = 0.05$; Table 5). Other main effects and interactions were nonsignificant, with the exception of replication. For blueback herring, only net type × net speed and net type × run time interactions were significant. For the 12.7-mm net, the catch was similar at each net speed; for the 24.0-mm net, it increased as speed changed from 0.6 to $1.0\,m\cdot s^{-1}$, but was similar at speeds of 1.0 and $1.4\,m\cdot s^{-1}$ (PSD = 0.84, $P = 0.05$; Fig. 2C). At $0.6\,m\cdot s^{-1}$, the catch by the 12.7-mm net was marginally greater than that for the 24.0-mm net. The net type × run time interaction was complex, but with one exception for each net type, the catch per cubic metre was similar at all run times (Fig. 2D).

The only significant differences in length were attributed to net speeds (Table 6). Lengths of alewives caught at 1.0 and $1.4\,m\cdot s^{-1}$ were similar but greater than those of fish taken at $0.6\,m\cdot s^{-1}$; lengths of blueback herring caught at 0.6 or $1.4\,m\cdot s^{-1}$ were similar, but less than those of fish caught at $1.0\,m\cdot s^{-1}$ as shown below:

	Net speed ($m\cdot s^{-1}$)		
	0.6	1.0	1.4
Alewife	54.2	63.2	63.2
Blueback	43.6	44.8	43.4

TABLE 4. Factorial, fixed effects analysis of variance, by net type, velocity, and run time, of the lengths of juvenile alewives and blueback herring collected by pushnet in the Mactaquac headpond, Saint John River, August 5, 6, and 9 (sums of squares adjusted for all design effects using weighted squares of means).

	Alewife				Blueback herring			
Source	df	MS	F	P	df	MS	F	P
Net type (A)	1	151.75	7.37	≪0.01	1	15.0	11.77	≪0.01
Net speed (B)	2	230.98	11.22	≪0.01	2	4.12	3.23	<0.05
Run time (C)	4	21.70	1.05	0.39	4	1.15	0.91	0.47
Replication	2	12.71	0.62	0.54	2	11.65	9.13	≪0.01
A × B	2	176.36	8.57	≪0.01	2	6.21	4.87	0.01
A × C	4	56.33	2.74	0.04	4	2.78	2.18	0.08
B × C	8	37.56	1.82	0.09	8	1.30	1.02	0.43
A × B × C	8	31.28	1.52	0.17	8	2.93	2.29	0.03
Error	51	20.59			56	1.28		
Total	83	216596.30			88	125860.96		

TABLE 5. Factorial, fixed effects analysis of variance, by net type, velocity, and run time, of $catch\cdot m^{-3}$ for juvenile alewives and blueback herring collected by pushnet in the Mactaquac headpond, Saint John River, September 12 and 13 (data transformed: $\log_e(X + 0.001)$).

		Alewife			Blueback herring		
Source	df	MS	F	P	MS	F	P
Net type (A)	1	0.0214	0.03	0.87	0.0008	0.01	0.98
Net speed (B)	2	51.3593	61.56	≪0.01	2.2764	1.71	0.19
Run time (C)	4	2.0064	2.40	0.06	1.7468	1.31	0.28
Replication	2	4.4490	5.33	0.01	3.3663	2.53	0.09
A × B	2	1.1722	1.41	0.25	4.5488	3.42	0.04
A × C	4	0.7389	0.89	0.48	4.2023	3.16	0.02
B × C	8	0.7855	0.94	0.49	1.9880	1.49	0.18
A × B × C	8	0.6925	0.83	0.58	1.8872	1.42	0.21
Error	58	0.8343			1.3316		
Total	89	2.0806			1.7125		

TABLE 6. Factorial, fixed effects analysis of variance, by net type, velocity, and run time, of the lengths of juvenile alewives and blueback herring collected by pushnet in the Mactaquac headpond, Saint John River, September 12 and 13 (sums of squares adjusted for all design effects using weighted squares of means).

	Alewife				Blueback herring			
Source	df	MS	F	P	df	MS	F	P
Net type (A)	1	29.60	0.89	0.35	1	22.63	2.49	0.12
Net speed (B)	2	465.53	13.95	≪0.01	2	36.17	3.99	<0.03
Run time (C)	4	47.63	1.43	0.24	4	14.02	1.54	0.21
Replication	2	33.55	1.01	0.37	2	6.99	0.77	0.47
A × B	2	175.74	5.27	≪0.01	2	32.57	3.59	<0.04
A × C	4	17.15	0.51	0.73	4	12.90	1.42	0.25
B × C	8	26.48	0.79	0.61	8	12.45	1.37	0.24
A × B × C	8	41.04	1.23	0.30	6[a]	9.83	1.08	0.39
Error	49	33.37			39	9.08		
Total	81	261070.38			69	36050.21		

[a]Redundant design effects assumed to be null.

For both species, the only significant interaction effects were for net type × net speed. Alewives caught at $0.6\,m\cdot s^{-1}$ by both net types were significantly smaller than those caught at 1.0 and $1.4\,m\cdot s^{-1}$ (Fig. 3). At $0.6\,m\cdot s^{-1}$, the 12.7-mm net caught longer alewives than did the 24.0-mm net. The longest blueback herring were caught at $1.0\,m\cdot s^{-1}$ by the 12.7-mm net.

During both August and September trials, the length frequen-

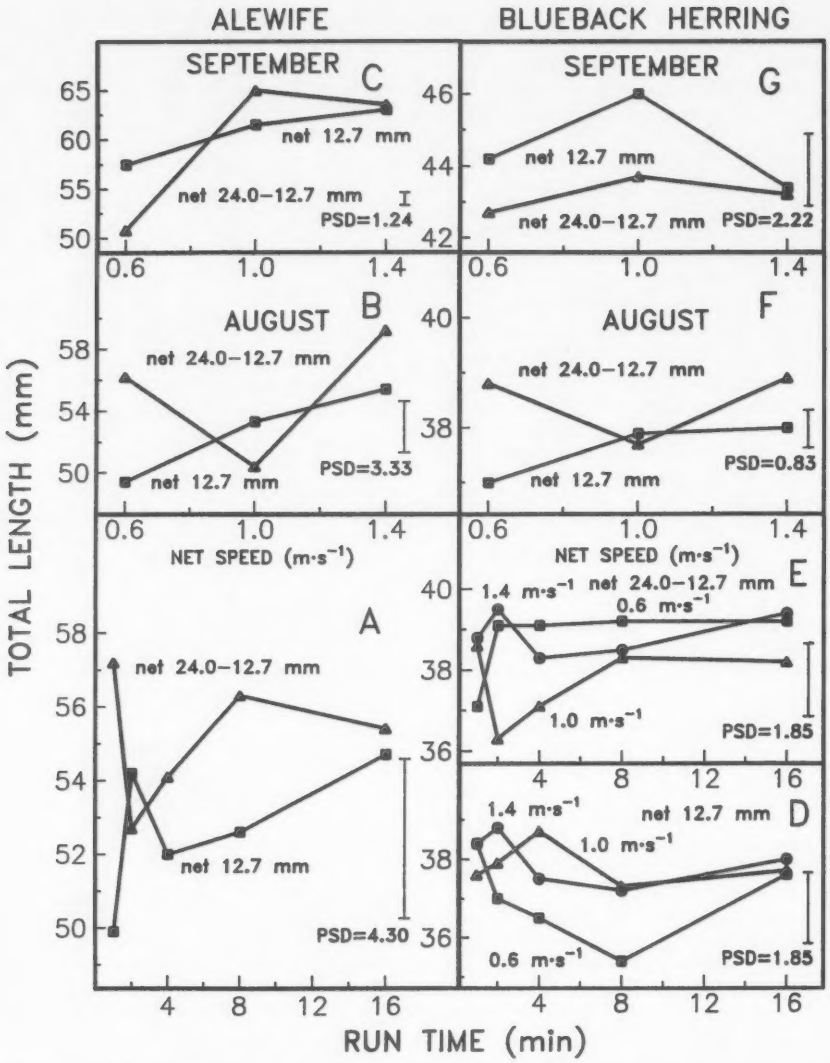


FIG. 3. Relationships between total length of juvenile alewives and (A) net type and run time (August), (B) net speed and net type (August), and (C) net type and net speed (September) and for blueback herring according to (D and E) net speed and run time by net type (August), (F) net type and net speed (August), (G) net speed (September), captured by pushnet in the Mactaquac Dam headpond, Saint John River.

cies of alewives caught by the 12.7-mm net were more normally distributed at all net speeds than were those for the 24.0-mm net (Fig. 4 and 5). In August, when alewives were relatively small, the catch length distributions of the 24.0-mm net were right skewed at $1.0\,m\cdot s^{-1}$ and left skewed at $1.4\,m\cdot s^{-1}$. Thus proportionately more longer alewives were captured and fewer shorter alewives retained at $1.4\,m\cdot s^{-1}$ than at $1.0\,m\cdot s^{-1}$.

Both nets produced strongly leptokurtic blueback herring length distributions. At all speeds, length distributions from the 12.7-mm net were right skewed and from the 24.0-mm net were left skewed. Alewife length distributions had greater ranges and were more skewed than were those of blueback herring. Alewife length ranges were similar for both nets but the 12.7-mm net retained a greater range of blueback herring lengths than did the 24.0-mm net. During September, both species were larger and the skewness of the length distributions was reduced for each species and net type.

To summarize, significant main effects for net type and net speed occurred for both catch and mean catch length for both species in August and, with one exception, for net speed in September. Interaction effects often differed between species. Seasonal decreases in abundance and increases in size of both

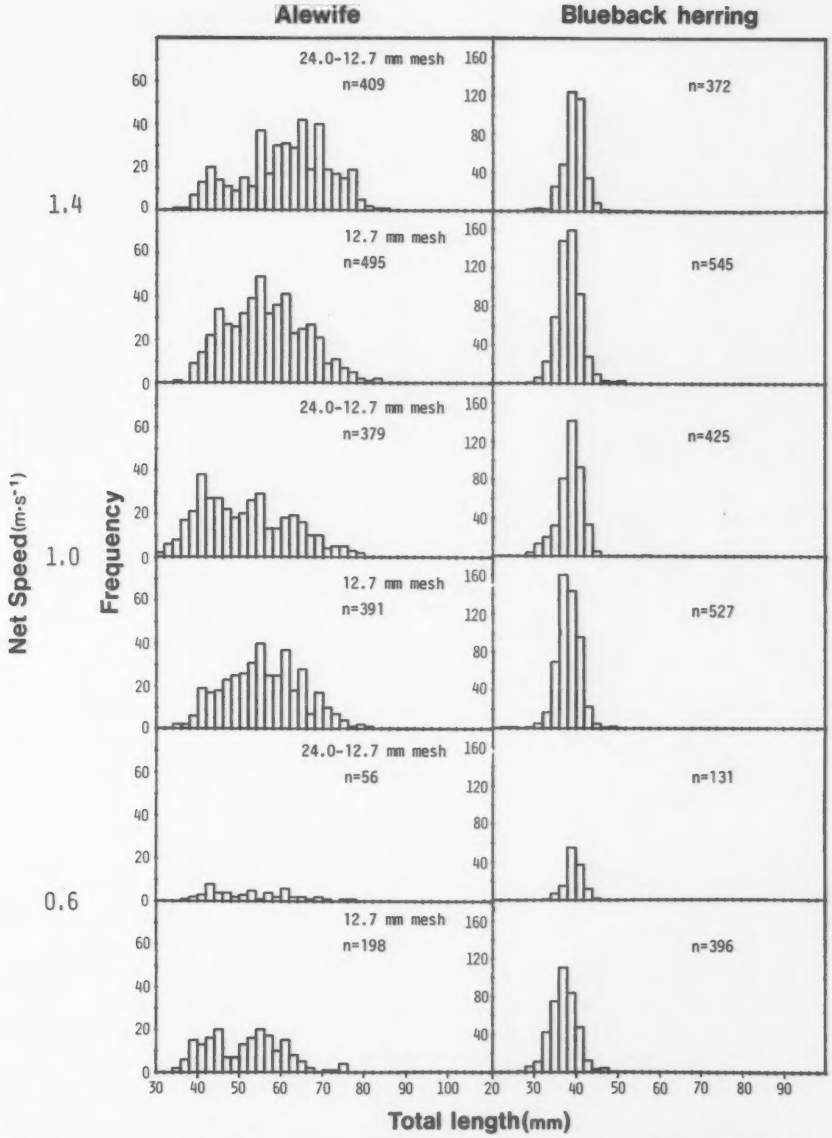


FIG. 4. Length frequency, by net type and net speed for all run times combined, of juvenile alewives and blueback herring collected by pushnet in the Mactaquac headpond, Saint John River, August 5, 6, and 9.

species resulted in fewer and generally less significant main and interaction effects. Both nets produced skewed and kurtotic length distributions, the degree of which was reduced as length increased.

At both high and low fish densities, the 12.7-mm net exhibited a curvilinear trend to increasing "precision" of the mean catch values, as measured by the coefficient of variation of the replicate runs, with increasing volume of water filtered. The turning point of the curve occurred between about 700 and 900 m^3 of filtrate, which corresponds to 5- to 7-min run times at 1.0 $m \cdot s^{-1}$. Increasing the run time from 5 to 8 min was estimated to increase the filtrate by 60% and precision by 35%. Longer run times produced little improvement. For the 24.0-mm net, precision remained similar with increasing filtrate.

For a given length, juvenile alewives are markedly deeper in the body than are blueback herring (Fig. 6). According to the "diagonal rule" (Smith et al. 1968), the minimum lengths for complete retention of alewives and blueback herring by a 12.7-mm stretched mesh (diagonal when open of 9.0 mm) are 49 and 53 mm, respectively, based on maximum body depth and 53 and 57 mm, respectively, based on depth at insertion of the pectoral fins.

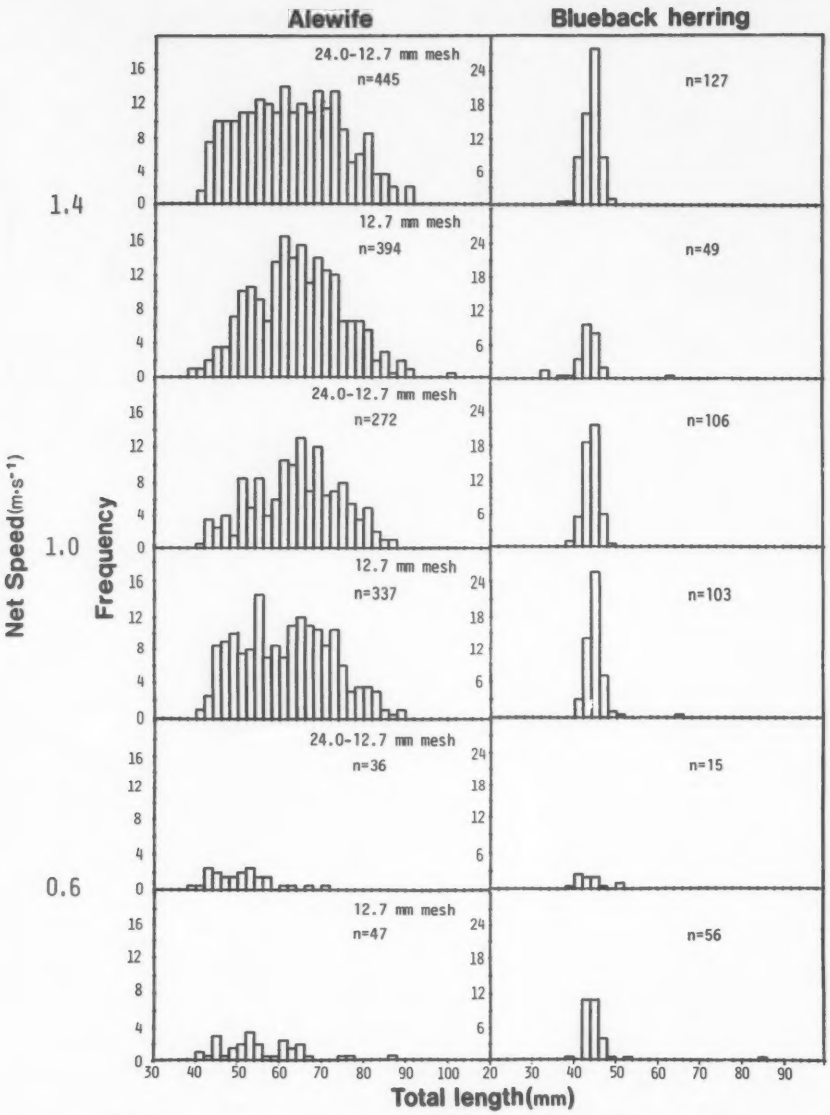


FIG. 5. Length frequency, by net type and net speed for all run times combined, of juvenile alewives and blueback herring collected by pushnet in the Mactaquac headpond, Saint John River, September 12 and 13.

Discussion

Much of the variability in catch per volume seems attributable to species-specific behavior and distribution because differences among net types, net speeds, and run times account for 49–74% of the observed variability. Since catch variability is typical of contagiously distributed organisms such as zooplankton and juvenile fish (Aron and Collard 1969; Turner et al. 1983), it is to be expected of juvenile alewives and blueback herring. The log-normal distribution fitted the catch data well. Care given to operational methods, including speed control, minimized the variability due to controllable factors, such as gear handling, and the use of a restricted sampling area and limited time frame, full darkness, calm water, and little current minimized the variability due to uncontrollable environmental factors. Both species are well dispersed throughout the lower headpond although within the trial area, between-zone (inshore and midstream) differences in density did occur but with no consistent pattern.

The choice of a constant K in the transformation relationship $\log_e (X + K)$ should be considered carefully. Values of 1.0 and 0.25 are common choices (Mosteller and Tukey 1977) and are suitable for a wide range of positive data values (Steel and Torrie 1980). However, for small numbers, particularly those between

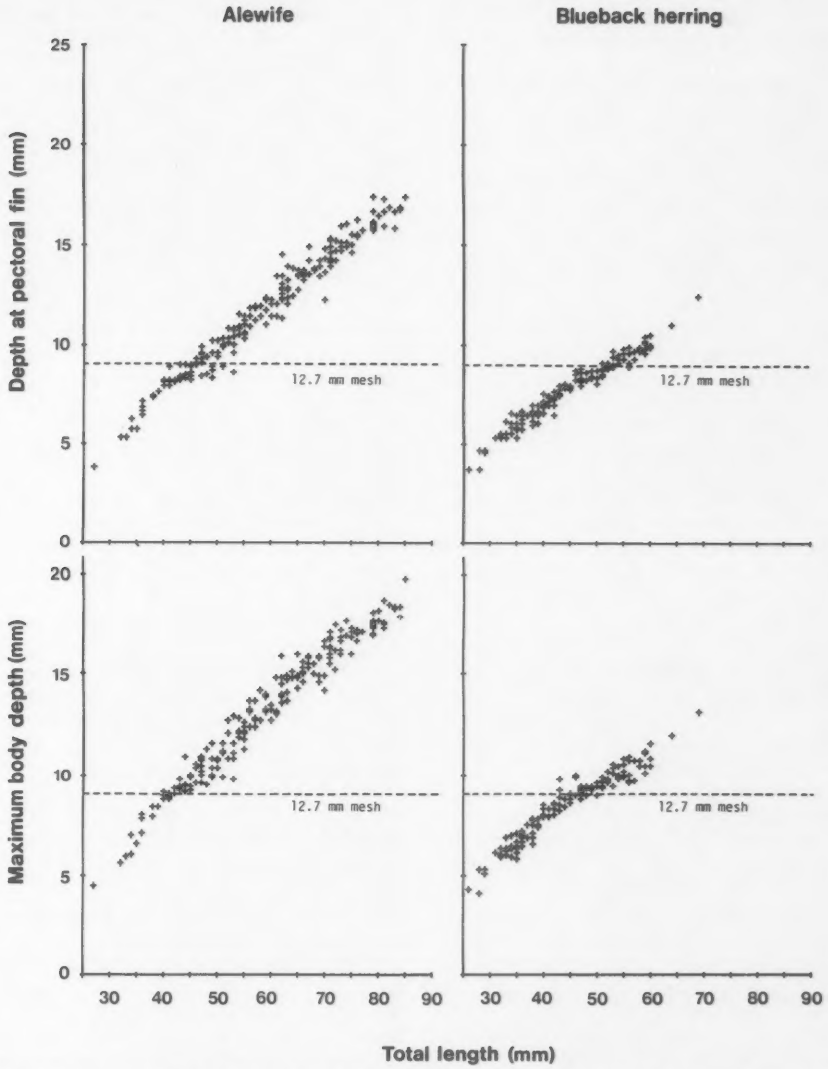


FIG. 6. Maximum body depth and depth at the pectoral fin in relation to total length of juvenile alewives and blueback herring collected by pushnet in the Mactaquac headpond, Saint John River.

zero and one, the common values of K may not achieve the goals of a transformation, i.e. additivity of environmental and treatment effects and residual errors normally and independently distributed with equal variance. Pennington and Grosslein (1978) found that the $\log_e (X + 1)$ transformation of groundfish trawl catches stabilized their variances but was less successful in reducing the nonnormality of the catch distribution. Both the level of significance and the sensitivity of F-tests to real departures from the null hypothesis in subsequent analyses of variance can be affected by departures from one or more of these assumptions (Steel and Torrie 1980), as was observed in trials using various values of K. The value of K should be such that $(X + K)$ is greater than zero for all observations yet not so large that it fails to remove all of the positive skew in the original data (Dunn and Clark 1974). Selection of an appropriate constant can be achieved by trying several values and comparing the results of plots and tests of each important criterion. Bartlett (1947) has suggested that the transformation $\log (X/(1 - X))$ may be useful where data are less than unity but no zero values are present. Snedecor and Cochran (1980, p. 295) noted that "the kinds of failures in the standard assumptions that are handled by transformation of the scale in which the data are analyzed" are such that "there is unlikely to be an ideal transformation for a given body of data."

No serious consequences are believed to result from violations of the assumptions underlying the analysis of variance methods employed. Logarithmic transformation of the catch per cubic metre data was found to satisfy adequately the require-

ments for ANOVA whereas the variance heterogeneity of the length data is expected to have minor consequences for the cell means ANOVA model.

Although the presence of significant interaction between factors might be considered reason to ignore the main effects, the practical importance of each factor is sufficient reason to examine them (Sokal and Rohlf 1969).

Net type (mesh composition) influenced the catch quantity and length composition early in the summer when alewives and blueback herring were relatively small, but had little effect in late summer when fish were larger. Over all speeds, a net of uniform mesh size (12.7 mm) more effectively caught both species than did a net with a cod-end of similar mesh size and a larger mesh body (24.0–12.7 mm), probably because the latter net permitted small fish to escape yet did not catch larger fish more effectively. Most fish escape through the after-part of the cod-end (Beverton 1963) but the catch length frequencies indicate that some small fish escaped through the body of the 24.0-mm net, probably during trawling but possibly during the haul out. The complex relationships between both catch and mean catch length and net speed for the net with two mesh sizes may result from larger fish more successfully avoiding the net at slower speeds, while smaller fish are more often extruded at higher speeds. These results support Tranter and Smith's (1968) conclusion that it is advisable to use nets of uniform mesh size because it simplifies filtration performance.

Although information on net selectivity is useful in evaluating the representativeness of sampling results, particularly when sampling over extended periods when selectivity might change with fish size (Aron and Collard 1969; Lenarz 1972), it may be less important when estimating an index of abundance, if it can be assumed constant among years.

Selection is not "knife-edged" and the retention pattern by length or girth typically exhibits a gradation in response to factors such as fish morphology, width and compressibility, and net speed and mesh flexibility (Pope 1966; Vannucci 1968). Although, according to the diagonal rule, juveniles 30–50 mm long may have been incompletely retained, a proportion, probably substantial, of the available fish were retained. Lenarz (1972) concluded that a net may completely retain larval fish that are less deep than the mesh diagonal, and Loesch and Kriete (1982) reported a median fork length of 25 mm (27 mm TL) for juvenile *Alosa* passing through the 12.7-mm cod-end of a 19.0-mm pushnet during covered cod-end trials. Only 18% of fish ≤27 mm (29 mm TL) passed through the cod-end. Although smaller mesh sizes have not been evaluated, a 12.7-mm mesh seems suitable for the quantitative capture of juvenile *Alosa* of lengths longer than about 30 mm to estimate a juvenile abundance index.

Net speed significantly influenced the catch abundance and length distributions of alewives and blueback herring. The relationship was curvilinear, with catch per cubic metre increasing as net speed increased. Other relationships may exist for other species (Aron and Collard 1969; Barkley 1972; Thayer et al. 1983). An asymptotic relationship might be expected as net speed approaches and then surpasses the avoidance ability of each species. However, the interaction of net type with net speed was such that only the 24.0-mm net exhibited a curvilinear catch–speed relationship; catch remained nearly constant for the 12.7-mm net. In addition to the reasons previously given, the advantages of a uniform mesh net include minimization of the effects of run speed on catch and length composition, particularly for smaller fish. Higher speeds altered the length distributions of the catch via the increased extrusion of small fish and by more efficient capture of larger fish, which are the dominant factors determining size distributions (Barkley 1972). When balancing the desirability of smaller mesh size or higher net speed, it should be recalled that, for a given mesh size, drag increases with the square of the net speed, thus requiring more propulsive power, increasing the strain on equipment, and increasing the potential for extrusion of catch. I suggest that, when using a 12.7-mm net, a speed of $1.0\,m \cdot s^{-1}$ or perhaps $1.2\,m \cdot s^{-1}$ would provide satisfactory estimates of the catch abundance and length distribution of juvenile alewives and blueback herring during their first summer of growth.

No evidence was found for net avoidance by either species in the size range encountered (<90 mm) at net speeds of 1.0 or $1.4\,m \cdot s^{-1}$. The capture of fish by trawls is affected by their maximum speed, manoeverability, endurance, and acceleration ability (Foster et al. 1981), information that seems unavailable for juvenile alewives and blueback herring. However, if fish between 50 and 100 mm long can be assumed to have maximum cruising speeds of $0.2–0.5\,m \cdot s^{-1}$ (based on herring data in Wardle 1977) and maximum (burst) swimming speeds about three times faster, then it seems reasonable that a net speed of $1.0–1.2\,m \cdot s^{-1}$ would capture and retain a high proportion of the juveniles encountered when a net with mouth dimensions of 1.5 × 1.5 m is used. Such a net is readily employed with boats sized for effective use in inland and estuarine waters where juvenile fish might be found, although other dimensions might prove optimum. It is desirable to use as large a sampling device as can be easily handled and moved at speeds appropriate to capture and retention of the target organism (Clutter and Anraku 1968). The bow-mounted pushnet has definite operational advantages for sampling juvenile pelagic fishes in near-surface waters (Kriete and Loesch 1980).

The minimal observed effect of run time on the catch abundance and length distribution is a consequence of the linear relationship between run time and catch (Schnack 1974; Pennington and Grosslein 1978). Selection factors (Pope 1966) did not increase with increasing run duration, and clogging was not a problem. Thus, the selection of an appropriate run time becomes an exercise in balancing field and laboratory operational cost and time requirements while obtaining adequate sample sizes and the desired precision of abundance estimates. Increasing the run time may increase the precision of catch estimates more than will increasing the net area (Wiebe 1972) and is perhaps more practical given the increased drag of a larger net size. For aggregated species, such as juvenile alewives and blueback herring, sample element size (run length) affects the precision of a density estimate little except at very low densities when more short tows may be slightly more precise than fewer longer tows (Lenarz and Adams 1980). Although it is desirable to increase run time at low densities (Pennington and Grosslein 1978), the simplicity of a single run time has merit when it is chosen to ensure adequate precision of catch estimates at the lowest expected densities. Only small increases in the precision of catch estimates occurred with run times exceeding 8 min when using the 12.7-mm net, but little change occurred at any run time when using the 24.0-mm net. A 5-min run time, as used by Kriete and Loesch (1980), appears practical and permits a catch sufficiently large for most purposes over the range of observed fish densities (geometric mean density range, based on 12.7-mm net catch: alewife, $0.017–0.048\ fish \cdot m^{-3}$; blueback herring, $0.005–0.242\ fish \cdot m^{-3}$). However, a 1- to 3-min increase in run time could provide a useful reduction in

sampling error if the time for survey completion is not unacceptably increased. Although this study provides no means to evaluate the relative influence of fish density and size on the occurrence and significance of main and interaction effects for net type, run speed, and run time, it seems reasonable that fish size is the major influence.

Significant replication effects occurred once for catch and once for length in two of eight ANOVA's. Given the high variability inherent in this type of data, additional replication would have been desirable but time constraints prevented this if each net trial was to be completed in one night.

The presence of interaction between net type, run speed, and run time in their effect on catch and length composition and the further influence of fish density, spatial distribution, and size on their manifestation requires caution when comparing and interpreting results from different studies. Standardization of the net design and operating procedure of the bow-mounted pushnet in the capture of juvenile *Alosa* would permit easier interpretation of results where interaction occurs between controllable factors, would facilitate the comparison of studies, and could prevent systematic bias between studies over time (Byrne et al. 1981; Gallagher and Conner 1983; Thayer et al. 1983). Environmental variability during and between surveys may influence survey results in an uncontrollable manner, yet the influence of factors such as wave, current, day/night, and seasonal conditions can be moderated by operational guidelines chosen to reduce their effect or based on known species behavior. The gear dimension and operating condition proposals advanced earlier will not eliminate biases caused by factors such as extrusion, avoidance, environmental conditions, or behavioral traits but should reduce some of the controllable variability, thereby improving the reliability and usefulness of juvenile *Alosa* surveys using pushnets.

Acknowledgements

I gratefully acknowledge the statistical advice and constructive review of the manuscript by J. Longmuir and S. J. Smith, the comments by two anonymous reviewers, the assistance of W. E. Anderson in the data analysis, and M. Worth, J. Hanusiak, and J. Smith in the field work.

References

ARON, W., AND S. COLLARD. 1969. A study of the influence of net speed on catch. Limnol. Oceanogr. 14: 242–249.

BARKLEY, R. A. 1972. Selectivity of towed-net samplers. Fish. Bull. 70(3): 799–820.

BARTLETT, M. S. 1947. The use of transformations. Biometrics 3: 39–52.

BEVERTON, R. J. H. 1963. Escape of fish through different parts of a cod-end. Spec. Publ. Int. Comm. Northw. Atlant. Fish. 5: 9–11.

BYRNE, C. J., T. R. AZAROVITZ, AND M. P. SISSENWINE. 1981. Factors affecting variability of research vessel trawl surveys, p. 258–273. *In* W. G. Doubleday and D. Rivard [ed.] Bottom trawl surveys. Can. Spec. Publ. Fish. Aquat. Sci. 58: 273 p.

CARROTHERS, P. J. G. 1981. Catch variability due to variations in groundfish otter trawl behavior and possibilities to reduce it through instrumented fishing gear studies and improved fishing procedures, p. 247–257. *In* W. G. Doubleday and D. Rivard [ed.] Bottom trawl surveys. Can. Spec. Publ. Fish. Aquat. Sci. 58: 273 p.

CLUTTER, R. I., AND M. ANRAKU. 1968. Avoidance of samplers, p. 57–76. *In* D. J. Tranter [ed.] Reviews on zooplankton sampling methods, Part I. UNESCO Monogr. Oceanogr. Methodol. 2, Zooplankton sampling.

COLTON, J. B., J. R. GREEN, R. R. BYRON, AND J. L. FRISELLA. 1980. Bongo net retention rates as affected by towing speed and mesh size. Can. J. Fish. Aquat. Sci. 37: 606–623.

DOUBLEDAY, W. G., AND D. RIVARD [ED.] 1981. Bottom trawl surveys. Can. Spec. Publ. Fish. Aquat. Sci. 58: 273 p.

DUNN, D. J., AND V. A. CLARK. 1974. Applied statistics: analysis of variance and regression. John Wiley & Sons, New York, NY. 387 p.

FOSTER, J. J., C. M. CAMPBELL, AND G. C. W. SABIN. 1981. The fish catching process relevant to trawls, p. 229–246. *In* W. G. Doubleday and D. Rivard [ed.] Bottom trawl surveys. Can. Spec. Publ. Fish. Aquat. Sci. 58: 273 p.

GALLAGHER, R. P., AND J. V. CONNER. 1983. Comparison of two ichthyoplankton sampling gears with notes on microdistribution of fish larvae in a large river. Trans. Am. Fish. Soc. 112: 280–285.

HULL, C. H., AND N. H. NIE. 1981. SPSS Update 7–9. McGraw-Hill Book Co., New York, NY. 402 p.

KRIETE, W. H. JR., AND J. G. LOESCH. 1980. Design and relative efficiency of a bow-mounted pushnet for sampling juvenile pelagic fishes. Trans. Am. Fish. Soc. 109: 649–652.

LEAMAN, B. M. 1981. A brief review of survey methodology with regard to groundfish stock assessment, p. 113–123. *In* W. G. Doubleday and D. Rivard [ed.] Bottom trawl surveys. Can. Spec. Publ. Fish. Aquat. Sci. 58: 273 p.

LENARZ, W. H. 1972. Mesh retention of larvae of *Sardinops caerulea* and *Engraulis mordax* by plankton nets. Fish. Bull. 70(3): 839–848.

LENARZ, W. H., AND P. B. ADAMS. 1980. Some statistical considerations of the design of trawl surveys for rockfish (*Scorpaenidae*). Fish. Bull. 78: 659–674.

LIPPSON, A. J., AND R. L. MORAN. 1974. Manual for the identification of early developmental stages of fishes of the Potomac River estuary. Md. Dep. Nat. Res. PPSR-MP-13: 282 p.

LOESCH, J. G. AND W. H. KRIETE, JR. 1982. Anadromous fisheries research, Virginia. Annual Report of the Virginia Institute of Marine Science, Gloucester Point, VA. 55 p.

LOESCH, J. G., W. H. KRIETE, JR., AND E. J. FOELL. 1982. Effects of light intensity on the catchability of juvenile anadromous *Alosa* species. Trans. Am. Fish. Soc. 111: 41–44.

MOSTELLER, F., AND J. W. TUKEY. 1977. Data analysis and regression. Addison-Wesley Publishing Co., Reading, MA. 588 p.

PENNINGTON, M. R., AND M. D. GROSSLEIN. 1978. Accuracy of abundance indices based on stratified random trawl surveys. I.C.E.S. Stat. Comm. C.M. 1978/D:13: 35 p.

POPE, J. A. 1966. Manual of methods for fish stock assessment Part 3. Selectivity of fishing gear. FAO Fish. Tech. Pap. 41: 41 p.

SCHNACK, D. 1974. On the reliability of methods for quantitative surveys of fish larvae, p. 201–212. *In* J. H. S. Blaxter [ed.] The early life history of fish. Springer-Verlag, New York, NY. 633 p.

SMITH, P. E., R. C. COUNTS, AND R. I. CLUTTER. 1968. Changes in filtering efficiency of plankton nets due to clogging under tow. J. Cons. Perm. Int. Explor. Mer 32: 232–248.

SNEDECOR, G. W., AND W. G. COCHRAN. 1980. Statistical methods. 7th ed. The Iowa State University Press, Ames, IA. 507 p.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. G. Freeman and Co., San Francisco, CA. 776 p.

STEEL, R. G. D., AND J. H. TORRIE. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Co., New York, NY. 633 p.

THAYER, G. W., D. R. COLBY, M. A. KJELSON, AND M. P. WEINSTEIN. 1983. Estimates of larval-fish abundance: diurnal variation and influences of sampling gear and towing speed. Trans. Am. Fish. Soc. 112: 272–279.

TRANTER, D. J., AND P. E. SMITH. 1968. Filtration performance, p. 27–56. *In* D. J. Tranter [ed.] Reviews on zooplankton sampling methods. Part 1. UNESCO Monogr. Oceanogr. Methodol. 2, Zooplankton sampling.

VANNUCCI, M. 1968. Loss of organisms through the meshes, p. 77–86. *In* D. J. Tranter [ed.] Reviews on zooplankton sampling methods, Part I. UNESCO Monogr. Oceanogr. Methodol. 2, Zooplankton sampling.

WARDLE, C. C. 1977. Effects of size on swimming speeds of fish, p. 299–313. *In* T. J. Pedley. [ed.] Scale effects in animal locomotion. Academic Press, New York, NY.

WIEBE, P. H. 1972. A field investigation of the relationship between length of tow, size of net and sampling error. J. Cons. Int. Explor. Mer 34: 268–275.

Biological Control of Excessive Phytoplankton Growth and the Enhancement of Aquacultural Production

Daniel Wilkins Smith

Department of Biological Sciences, University of California, Santa Barbara, CA 93106, USA

Smith, D. W. 1985. Biological control of excessive phytoplankton growth and the enhancement of aquacultural production. Can. J. Fish. Aquat. Sci. 42: 1940–1945.

A method is proposed to control phytoplankton biomass in aquacultural ponds, using both zooplankton and filter-feeding silver carp (*Hypophthalmichthys molitrix*). The technique maintains co-existence of zooplankton and filter-feeding fish by excluding the fish from part of the water column. Zooplankton, which feed on smaller algal species, and silver carp, which feed on large algae and zooplankton, together can consume all sizes of phytoplankton, thus controlling algal biomass. This technique was tested in 1000-L tanks, some containing channel catfish (*Ictalurus punctatus*) alone, some both catfish and silver carp, and others catfish and silver carp with a zooplankton refuge. The refuge permitted coexistence of high densities of large zooplankters with the filter-feeding fish. This combination of filter-feeders reduced algal biomass by as much as 99%, increased phytoplankton diversity, and showed a trend toward improved silver carp growth compared with treatments without a refuge. The proposed technique could be applied to both intensive and extensive aquacultural systems.

On propose une méthode de contrôle de la biomasse du phytoplancton dans les étangs aquicoles, méthode qui fait appel au zooplancton et à la carpe argentée, (*Hypophthalmichthys molitrix*) qui se nourrit par filtration. La technique permet la coexistence du zooplancton et de poissons filtreurs en écartant les poissons d'une partie de la colonne d'eau. Le zooplancton, qui se nourrit d'espèces d'algues de petite taille, et la carpe argentée, qui s'alimente d'algues plus grosses et de zooplancton, peuvent consommer ensemble des organismes phytoplanctoniques de toutes les tailles, permettant ainsi de contrôler la biomasse des algues. Cette technique a été mise à l'essai dans des réservoirs de 1000 L, certains contenant seulement des barbues de rivière (*Ictalurus punctatus*), d'autres des barbues de rivière et des carpes argentées et d'autres encore des barbues de rivière et des carpes argentées ainsi qu'un abri pour le zooplancton. L'abri a permis la coexistence de densités élevées de gros organismes zooplanctoniques et de poissons filtreurs. L'utilisation de cet assortiment de filtreurs a permis de réduire d'autant que 99 % la biomasse des algues et d'accroître la diversité des organismes phytoplanctoniques et a favorisé la croissance des carpes argentées comparativement aux résultats obtenus lors d'expériences où l'on n'avait pas utilisé d'abri. La technique proposée pourrait s'appliquer aux systèmes aquicoles aussi bien intensifs qu'extensifs.

Received March 3, 1985
Accepted August 6, 1985
(J8152)

Reçu le 3 mars 1985
Accepté le 6 août 1985

This paper introduces a new technique to control phytoplankton biomass biologically, using a combination of filter-feeding fish and zooplankton. Excessive phytoplankton growth is a problem, because it may negatively affect water quality in fish ponds in at least three ways. First, it can lead to chronic nighttime oxygen deficits, since algae are the major oxygen consumers in the pond as well as the producers of detritus that is broken down by oxygen-consuming bacteria (Boyd 1973; Boyd et al. 1978). Second, dense algal blooms collapse periodically; subsequent decomposition of the dead algae can cause costly fish kills due to anoxia (Boyd et al. 1975; Barica 1975, 1978) and due to toxic ammonia levels (Seymour 1980; Tucker et al. 1984). Excess ammonia can kill fish even in ponds that are constantly aerated (Shilo and Rimon 1982). Third, dense algal blooms can produce chemicals that ruin the flavor of fish (Lovell and Sackey 1973; Brown and Boyd 1982). Thus, dense populations of algae can be a major impediment to the maintenance of adequate water quality in both extensive and intensive aquacultural systems (Anonymous 1975; Boyd 1982; Sin and Chiu 1982; Costa-Pierce et al. 1985).

Previous attempts to control algal growth have relied primarily on two techniques: use of herbicides and restriction of nutrient inputs (Anonymous 1975; Boyd 1982). Herbicides are expensive, difficult to administer properly, and potentially damaging to the environment (Janik et al. 1980). Moreover, their use may be counterproductive, because they often stimulate phytoplankton die-offs (Tucker and Boyd 1978). Restriction of nutrient inputs is accomplished by withholding feed and/or by reduction of fertilization — both of which are incompatible with maximizing fish production. Other expedients (flushing, constant aeration) are expensive and not completely effective (Boyd 1982; McGee and Boyd 1983).

Attempts to control phytoplankton using filter-feeding fish alone have produced inconclusive results (Janusko 1974, 1978; Pretto-Malca 1976; Dunseth 1977; Costa-Pierce et al. 1985). In some of these studies, algal biomass actually increased when algae-eating fish were stocked. The explanation for this counter-intuitive result may simply be that the fish are ineffective as control agents, because they consume only large algal species (Drenner et al. 1984; Smith 1986). Silver carp (*Hypophthalmich-*

thys molitrix), for example, have reduced selectivity for particles smaller than 40 μm and cannot filter particles smaller than 9 μm (Smith 1986). Because filter-feeding fish also consume zooplankton (Kajak et al. 1975; Grygierek 1978), which feed on phytoplankton less than 30 μm in diameter (Kajak 1979; McCauley 1983; Vyhnalek 1983), the fish simultaneously remove the predators of the smaller algal species as well as their large-algae competitors (Opuszinski 1972; Kajak 1979). Small algal species can then flourish at noxious levels. Thus, attempts to control biomass using fish alone will probably succeed only in controlling the size structure of the algae.

Together, fish and zooplankton have complementary feeding niches that span all sizes of phytoplankton; thus, the two types of filter-feeders should be able to control algal biomass. Unfortunately, they do not co-occur at densities adequate to enforce control, because predation by the fish greatly reduces zooplankton densities. The technique described herein resolves this dilemma by providing the zooplankton with a refuge from fish predation.

The experiment tested three predictions: (1) a refuge will allow high densities of zooplankton to coexist with filter-feeding fish; (2) a combination of filter-feeding fish and zooplankton can control algal biomass; (3) the filter-feeding fish will grow better when a refuge prevents them from exterminating their prey.

Methods

Six cylindrical, translucent fiberglass tanks (each 1.3 m high and 1 m in diameter) were divided into three treatments (Table 1). The tanks were filled with water from a common source that 2 mo earlier had been fertilized heavily and inoculated with algae from several local lakes and ponds. Water volume in the tanks was maintained at 1000 L.

The first treatment, which contained only channel catfish (*Ictalurus punctatus*), corresponds to an intensive aquacultural operation with no algal control. The second treatment, which contained catfish and silver carp, corresponds to an intensive pond in which biological control of algae is attempted with filter-feeding fish alone. The third treatment, in which fish were excluded from one half of the water column, represents a system in which algae is controlled using both filter-feeding fish and zooplankton. At stocking, the mean standard length of catfish was 12.68 cm and of silver carp was 10.51 cm.

Catfish were fed a high-protein commercial trout chow at a rate approximating 2% of body weight per day. Their excreta and uneaten food served as a natural source of nutrients for phytoplankton growth.

In tanks with a refuge, all fish were excluded from one half of the tank by partitions of Vexar® plastic mesh (1.5 × 1.5 cm pore size). To control for any effects of the mesh, partitions of identical construction and materials were placed in the other tanks. These control partitions, however, had large holes cut in the mesh that allowed passage of fish.

Equal subsamples of a zooplankton slurry taken from a variety of local sources were introduced into the tanks. Silver carp and zooplankton were introduced immediately after filling on April 25, 1983, and catfish fingerlings were stocked 2 wk later on May 5. The experiment ended on June 15, 1983. Tanks were stirred thoroughly at least once weekly. Water temperatures stayed at 20 ± 3°C.

On each sampling date, water samples were taken in duplicate from opposite sides of each tank, for a total of four samples per tank. To produce each sample, 1800 mL was siphoned from three strata in the tank (in the middle of the water column and about 10 cm from the bottom and top) using a hose (2 cm, ID) that was connected to a metre stick. All 5400 mL was then pooled in a bucket, producing one integrated water sample. Subsequently, one zooplankton and one algae subsample were taken from the bucket. Zooplankton subsamples of 1800 mL were strained through 80-μm mesh, and the organisms were preserved in iced, sugared formalin (Haney and Hall 1973). Whole-water algae subsamples were preserved with Lugol's solution, and the phytoplankton were counted using the Utermöhl technique (Lund et al. 1958). On the last three dates only, subsamples were also taken for estimation of chlorophyll *a*. These were filtered with Whatman GF/C filters, extracted with acetone, and corrected for phaeophytin (Strickland and Parsons 1969).

TABLE 1. Experimental design. Control agents are the expected consumers of phytoplankton in the tanks. No data could be found to predict when catfish become primarily benthic feeders, so no clear prediction could be made for the treatment with catfish only. CC = catfish only; CSC = catfish and silver carp; RE = refuge treatment.

Treatment code	Catfish	Silver carp	Refuge	Biocontrol agents
CC	10	0	No	Zooplankton?
CSC	10	11	No	Silver carp
RE	10	11	Yes	Silver carp, zooplankton

Filtration rates of zooplankton were calculated using values found in Haney (1973). Observed densities of *Ceriodaphnia* were multiplied by 4.6 mL/d, densities of *Daphnia* by 6.4 mL/d, and densities of *Chydoridae* by 0.18 mL/d. *Simocephalus* and *Scapholeberis* were assigned filtration rates of cladocerans of similar size (i.e. *Simocephalus* was assigned the value for *Daphnia*, and *Scapholeberis* was assigned that of *Ceriodaphnia*). Each copepod nauplius, which averaged one tenth the biomass of a chydorid, was assigned a value of 0.018 mL/d. Cyclopoid copepod adults and copepodites were ignored in these calculations, because they are primarily raptorial carnivores. Note that since zooplankton filtration rates vary with taxa, body size, and environmental conditions (Peters and Downing 1984), these values should be used only as a relative index of zooplankton filtration.

Results

For the proposed algal control technique to succeed, the refuge must protect the zooplankton from predation by filter-feeding fish. This condition was satisfied. The refuge affected both the numbers and species composition of the zooplankton communities (Fig. 1). Tanks with silver carp and no zooplankton refuge were dominated by small chydorid cladocerans and cyclopoid copepods. Refuge tanks were dominated by large cladocerans, mostly *Ceriodaphnia*, *Daphnia*, and occasionally *Simocephalus* and *Scapholeberis*. Zooplankton response in the two catfish-only tanks were highly variable. In one tank, the zooplankton were dominated by chydorids, but the other had the highest *Ceriodaphnia* densities of any tank.

Grazing by zooplankton within the catfish-only treatment, while highly variable between tanks, was similar to that of the refuge treatment (Fig. 2). Since zooplankton body size dramati-

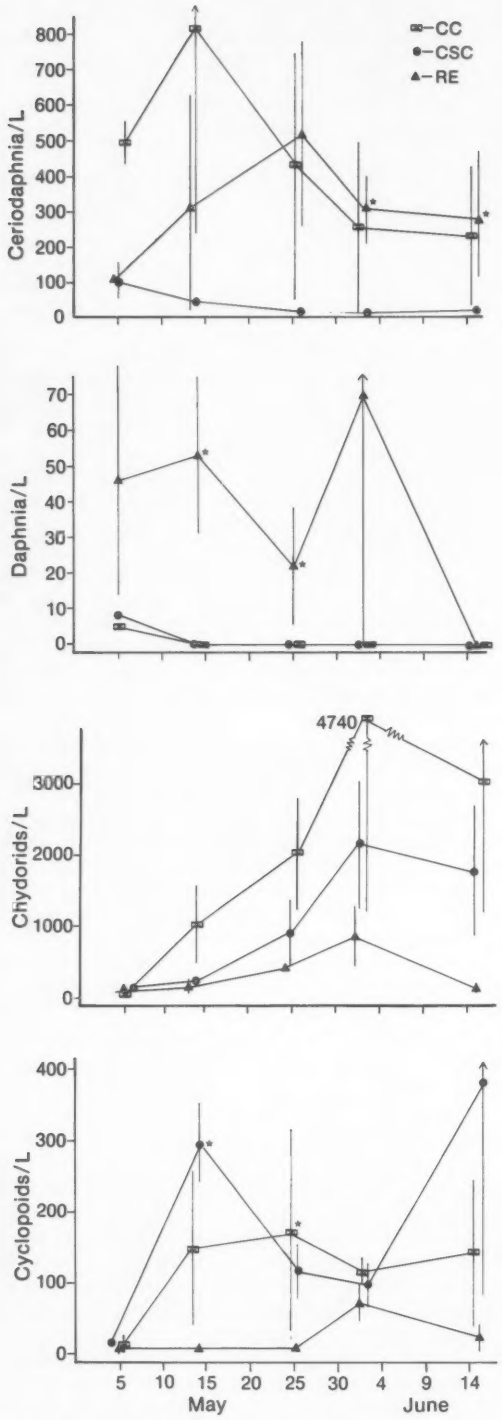


FIG. 1. Abundance of major zooplankton taxa (means ± SE). With two replicates, the mean plus and minus the standard error equals the range. CC, catfish-only treatment; CSC, treatment with catfish and silver carp; RE refuge treatment; *denotes dates on which the RE treatment differed from the CSC treatment (t-test, $*p < 0.05$).

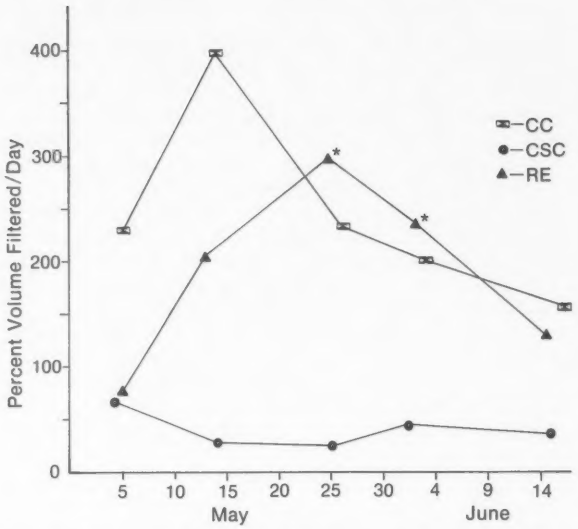


FIG. 2. Zooplankton community filtration rates. Treatment codes as defined in Fig. 1; *denotes dates on which RE treatment differed from the CSC treatment (t-test, $p < 0.05$).

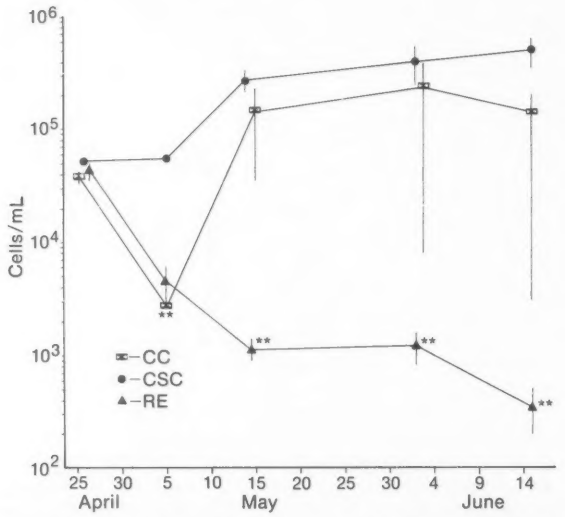


FIG. 3. Phytoplankton density (means ± SE). Treatment codes as defined in Fig. 1; **denotes dates on which there was a significant effect of treatment (ANOVA, $p < 0.01$).

cally affects filtration rates, the zooplankton in refuge tanks had considerably higher calculated filtration rates than nonrefuge tanks with silver carp (Fig. 2). This effect, combined with the effect of silver carp grazing, dramatically reduced algal biomass to as little as 0.01 the biomass in control treatments (Fig. 3; Table 2). These results were highly significant from the second week of the experiment. Catfish-only tanks also had less phytoplankton than nonrefuge tanks with silver carp, but this effect was not significant (Fig. 3).

The majority of zooplankton in refuge tanks were found on the side from which fish were excluded. On average, the zooplankton filtration rates within the refuge were three times

TABLE 2. Chlorophyll *a* values (means ± SE). CC is the catfish-only treatment, CSC is the treatment with catfish and silver carp, and RE is the refuge treatment. PROB refers to the significance level (ANOVA) of log-transformed values. % reduction is the average reduction of chlorophyll *a* compared with nonrefuge treatments, i.e. % reduction = RE/(CSC + CC)/2.

	May 25	June 2	June 15
CC	548±76	677±43	721±220
CSC	766±222	883±201	1460±263
RE	24±6.5	25±8.0	11±1.7
PROB	0.009	0.007	0.001
% reduction	96	97	99

that of the side with fish. Side to side differences in the zooplankton communities of the nonrefuge treatments were always minor. The proportion of zooplankton filtration on one side showed a significant effect of treatment ($p < 0.05$, ANOVA).

Silver carp grew 40% faster in refuge tanks compared with nonrefuge tanks. Average change in length was 1.41 cm for the refuge treatment compared with 1.01 for fish in the nonrefuge treatment, but this effect was not significant.

Discussion

The algal control technique produced a large-scale reduction in algal biomass. At least three hypotheses could explain this result. (1) The fish could have stirred the sediments, reducing light and phytoplankton growth (Kajak 1979). This hypothesis can be eliminated, since the refuge treatments were very clear. (2) The grazers could have reduced nutrients available to the phytoplankton by incorporating these nutrients into their own biomass. This hypothesis is also not likely, because catfish were fed high-protein pellets, a substantial component of which remains as unassimilated nutrients after feeding. Using data from Boyd (1982) for phosphorus content of fish food and nutrient assimilation of catfish, feeding contributed 1.37 g of phosphorus to each tank over the duration of the experiment. In addition, minimum excretion rates of *Ceriodaphnia* alone would have contributed approximately 3.7 mg of phosphorus per day to the refuge tanks (Peters 1975). These values are about 340 and 190 times, respectively, the dangerous loading level for lakes (Vollenweider 1968). Thus, excess nutrients were available throughout the experimental period. (3) The fish and zooplankton could have controlled algal biomass by constant grazing on all sizes of phytoplankton.

The results of this experiment substantiate the third hypothesis and indicate that neither fish grazing nor zooplankton grazing alone can control algal biomass. The similarity of zooplankton filtration rates in the refuge and catfish-only treatments permits use of these two treatments to assess the effect of adding fish filtration onto a relatively constant zooplankton grazing pressure. Likewise, both treatments with silver carp have similar fish grazing, so these two treatments can be used to estimate the effect of adding zooplankton to a constant fish filtration. Neither zooplankton nor fish alone were apparently sufficient to control algal biomass, while both together were very successful.

This work tacitly assumes that phytoplankton escape predation solely or primarily by being too big or too small. This assumption is an oversimplification, because algae have other survival strategies, primarily toxicity (Lampert 1981) and indigestibility (Porter 1977). These might present problems for this technology, but there is cause for optimism. Filter-feeding fish feed on species of algae that are associated with toxicity (Moriarty 1973; Hamada et al. 1983), and the fish produce a heavy fecal pellet that could remove indigestible algae from the water column. Moreover, certain species of zooplankton consume and thrive on toxic algae (Peter Starkweather, University of Nevada at Las Vegas, pers. comm.). Thus, other survival strategies of the algae may not pose serious problems to algal control with fish and zooplankton.

The results of this trial support this optimism. The severe grazing pressure found in refuge tanks would seem opportune for the dominance of species that are indigestible or toxic; but this did not occur. The phytoplankton of these tanks did contain several taxa associated with toxicity or indigestibility, notably *Anabaena*, *Mycrocystis*, and *Gloeocystis*; however, these taxa never became common.

The growth of silver carp illustrates several important points. First, the fish did grow during the experiment; therefore, the technique functions at stocking rates low enough to allow growth of the silver carp. One can grow fish as well as control algal growth. Second, increased growth in the refuge treatment bodes well for this technique, because I made no attempt to maintain prey at levels optimal for silver carp growth. Third, even if one takes a more conservative view of the data, silver carp in refuge tanks grew no worse in spite of being restrained from one half of the water column. Thus, the algal control technique will not reduce the growth of the filter-feeding fish. Lastly, reduced growth of silver carp in the nonrefuge treatment amidst 10–100 times the algal biomass illustrates, as suggested by Opuszinski (1978) and Schroeder (1978), that algal biomass, irrespective of size composition, is a poor indicator of growth conditions for silver carp. This last observation also supports the hypothesis that the supposedly herbivorous silver carp grows better on an omnivorous diet (Bitterlich and Gnaiger 1984).

Integrated algal control with fish and zooplankton might also redress the problem of algal die-offs. Theoretical and empirical evidence suggests that highly diverse algal communities are less likely to collapse than blooms dominated by one species (Smith 1985). The refuge system could promote species diversity of the algae, because different kinds of predation pressure will occur on either side of the fish barrier (e.g. primarily fish grazing on one side, zooplankton grazing within the refuge). Refuge tanks did have significantly higher phytoplankton diversity (Fig. 4).

This technique could be applied to both intensive and extensive aquacultural systems. Intensive systems function as feedlots. Fish are fed a nutritionally adequate diet; thus, they need not rely on production of natural foods within the ponds (Brown and Gratzek 1980). Here, the major limitation on production is water quality, especially the level of dissolved oxygen (Andrews et al. 1973). Integrated algal control with zooplankton and fish could improve water quality in intensive aquacultural systems troubled by excessive phytoplankton. Moreover, the control agents are either edible fish or organisms readily consumed by fish. Thus, the technique could convert a serious pest into a valuable resource.

The technique could also be applied to extensive aquacultural systems, which are sometimes plagued by excess algae (Anonymous 1975; Sin and Chiu 1982). Besides controlling algal biomass, the refuge system could increase fish production directly by more efficient use of algae. Extensive aquacultural systems function as pasturages; stocked fish harvest natural

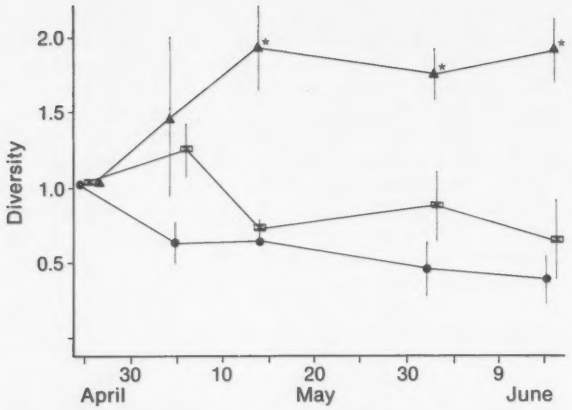


FIG. 4. Phytoplankton diversity (means ± SE). Shannon–Weiner diversity, $\Sigma p_i \log p_i$. Treatment codes as defined in Fig. 1; *denotes dates on which there was a significant effect of treatment (ANOVA, $p < 0.05$).

foods produced within the pond ecosystem. Because phytoplankton are the ultimate source of most natural production (Anonymous 1975), they constitute a major resource to extensive aquaculture. Thus, very dense algal blooms are problematic not only for the water quality problems discussed above, but also because they represent an underutilized resource. Extensive systems with filter-feeding fish, like those intensive systems in which filter-feeding fish have been tried as biocontrol agents, are driven to states in which all or most of the primary production is too small to be consumed directly by the fish (Schroeder 1978). A refuge system could maintain the trophic link between fish and primary production by preventing the fish from overexploiting the large algae and zooplankton. This polyculture of fish and fish food (the zooplankton) could potentially control problems with excessive algal growth while enhancing the efficiency of fish production.

Acknowledgements

This work was funded by grants from the University of California Appropriate Technology Office and the Northern Mfg. Co. The Vexar® plastic mesh was graciously supplied by AMOCO Fabrics of Atlanta, Georgia. I thank Scott Cooper, Edward McCauley, James Bence, Ruth Anderson, John Melack, and Jeff Janik for their help.

References

ANDREWS, J. W., T. MURAI, AND G. GIBBONS. 1973. The influence of dissolved oxygen on the growth of channel catfish. Trans. Am. Fish. Soc. 102: 835–838.

ANONYMOUS. 1975. Lecture notes on the training course in fresh-water fish culture. III. Adult fish culture in pond. Kwangtung Provincial Research Institute of Aquatic Products, Kwangchow, China.

BARICA, J. 1975. Collapse of algal blooms in prairie pothole lakes: their mechanism and biological impact. Verh. Int. Ver. Limnol. 19: 606–615.

1978. Collapses of *Aphanizomenon flos-aquae* blooms resulting in massive fish kills in eutrophic lakes: effect of weather. Verh. Int. Ver. Limnol. 20: 208–213.

BITTERLICH, G. AND E. GNAIGER. 1984. Phytoplanktivorous or omnivorous fish? Digestibility of zooplankton by silvercarp. Aquaculture 40: 261–263.

BOYD, C. E. 1973. The chemical oxygen demand of waters and biological material from ponds. Trans. Am. Fish. Soc. 102: 606–611.

1982. Water quality management for pond fish culture. Elsevier Sci. Publ., New York, NY. 318 p.

BOYD, C. E., E. E. PRATHER, AND R. W. PARKS. 1975. Sudden mortality of a massive phytoplankton bloom. Weed Sci. 23: 61–67.

BOYD, C. E., R. P. ROMAIRE, AND E. JOHNSTON. 1978. Predicting early morning dissolved oxygen concentrations in channel catfish ponds. Trans. Am. Fish. Soc. 107: 484–492.

BROWN, E. E., AND J. R. GRATZEK. 1980. Fish Farming Handbook. AVI Publishing Co., Inc., Wesport, CT. 350 p.

BROWN, S. W., AND C. E. BOYD. 1982. Off-flavor in channel catfish from commercial ponds. Trans. Am. Fish. Soc. 111: 379–383.

COSTA-PIERCE, B. A., S. MALECHA, AND E. LAWS. 1985. Effects of fish and plant polyculture and feed source on the water quality of prawn (*Macrobrachium rosenbergii*) ponds in Hawaii. Trans. Am. Fish. Soc. (In press)

DRENNER, R. W., S. B. TAYLOR, X. LAZZARO, AND D. KETTLE. 1984. Particle-grazing and plankton community impact of an omnivorous cichlid. Trans. Am. Fish. Soc. 113: 397–402.

DUNSETH, D. R. 1977. Polyculture of channel catfish, *Ictalurus punctatus*, silver carp, *Hypophthalmichthys molitrix*, and three all male tilapias, *Sarotherodon* spp. Ph.D. thesis, Auburn University, Auburn, AL.

GRYGIEREK, E. 1978. The influence of silver carp on eutrophication of the environment of common carp ponds. IV. Zooplankton. Rocz. Nauk Roln. Ser. H Rybactwo 99: 81–90.

HAMADA, A., W. MAEDA, J. IWASAKI, AND A. KUMAMARU. 1983. Conversion efficiency of phytoplankton in the growth of silver carp, *Hypophthalmichthys molitrix*. Jpn. J. Limnol. 44: 321–328.

HANEY, J. F. 1973. An in-situ examination of the grazing activities of natural zooplankton communities. Arch. Hydrobiol. 72: 87–132.

HANEY, J. F., AND D. J. HALL. 1973. Sugar coated *Daphnia*: a preservation technique for cladocera. Limnol. Oceanogr. 18: 331–333.

JANIK, J. J., W. D. TAYLOR, AND J. W. BARKO. 1980. A compilation of common algal control and management techniques. No. AD-A082013. Available from the National Technical Information Service, Springfield, VA.

JANUSKO, M. 1974. The effect of three species of phytoplanktivorous fish on algae development. Pol. Arch. Hydrobiol. 21: 431–454.

1978. The influence of Silver Carp (*Hypophthalmichthys molitrix*) on eutrophication of the environment of carp ponds. III. Phytoplankton. Rocz. Nauk Roln. Ser. H Rybactwo 99: 55–79.

KAJAK, Z. 1979. The possible use of fish, especially silver carp, *Hypophthalmichthys molitrix*, to overcome water blooms in temperate water bodies. *In* J. Salanki and P. Biro [ed.] Human impacts on life in fresh waters. Symp. Biol. Hung. 19: 77–86.

KAJAK, Z., J. I. RYBACK, I. SPODNIEWSKA, AND W. GODLESSKI-LIPOWA. 1975. Influence of the planktivorous fish, *Hypophthalmichthys molitrix*, on the plankton and benthos of the eutrophic lake. Pol. Arch. Hydrobiol. 22: 301–310.

LAMPERT, W. 1981. Inhibitory and toxic effects of blue-green algae on *Daphnia*. Int. Rev. Gesamten Hydrobiol. 66: 285–298.

LOVELL, R. T., AND L. A. SACKEY. 1973. Absorption by channel catfish of earthy–musty flavor compounds synthesized by cultures of blue-green algae. Trans. Am. Fish. Soc. 102: 774–777.

LUND, W. G., C. KIPLING, AND E. D. LECREN. 1958. The inverted microscope method of estimating algal number and the statistical basis of estimating by counting. Hydrobiologia 11: 143–170.

MCCAULEY, E. 1983. The impact of zooplankton on the dynamics of natural phytoplankton populations. Ph.D. thesis, McGill University, Montreal. Que.

MCGEE, M. V., AND C. E. BOYD. 1983. Evaluation of the influence of water exchange in channel catfish ponds. Trans. Am. Fish. Soc. 112: 557–560.

MORIARTY, D. J. 1973. The physiology of digestion of blue-green algae in the cichlid fish, *Tilapia nilotica*. J. Zool. Lond. 7: 25–39.

OPUSZINSKI, K. 1972. Use of phytophagious fish to control aquatic plants. Aquaculture 1: 61–73.

1978. The influence of the silver carp (*Hypophthalmichthys molitrix*) on eutrophication of the environment of carp ponds. Part VII. Recapitulation. Rocz. Nauk Roln. Ser. H Rybactwo 99: 127–150.

PETERS, R. H. 1975. Phosphorus regeneration by natural populations of limnetic zooplankton. Verh. Int. Ver. Limnol. 19: 273–279.

PETERS, R. H., AND J. A. DOWNING. 1984. Empirical analysis of zooplankton filtering and feeding rates. Limnol. Oceanogr. 29: 763–784.

PORTER, K. G. 1977. The plant–animal interface in freshwater ecosystems. Am. Sci. 65: 159–170.

PRETTO-MALCA, R. 1976. Polyculture systems with channel catfish as the principal species. Ph.D. thesis. Auburn University, Auburn, AL. 202 p.

SCHROEDER, G. L. 1978. Autotrophic and heterotrophic production of microorganisms in intensively-manured fish ponds, and related fish yields. Aquaculture 14: 303–325.

SEYMOUR, E. A. 1980. The effect and control of algal blooms in fish ponds. Aquaculture 19: 55–74.

SHILO, M., AND A. RIMON. 1982. Factors which affect the intensification of fish breeding in Israel. II. Ammonia transformation in intensive fish ponds. Bamidgeh 34: 101–113.

SIN, A. W., AND M. T. CHIU. 1982. Summer and winter kills in fish ponds of Hong Kong and their possible predictions. Aquaculture 29: 125–135.

SMITH, D. W. 1985. Biological control of excessive phytoplankton growth and enhancement of aquacultural production. Ph.D. thesis, University of California, Santa Barbara, CA.

1986. The feeding ecology of silver carp. Pro. 2nd Int. Conf. Warm Water Aquaculture — Finfish, Laie, Hawaii, 1985. AVI Publishing Co., Inc., Westport, CT. (In press)

STRICKLAND, J. D. H., AND T. R. PARSONS. 1969. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167: 311p.

TUCKER, S. S., AND C. E. BOYD. 1978. Consequences of periodic application of copper sulfate and simazine for phytoplankton control in catfish ponds. Trans. Am. Fish. Soc. 107: 316–320.

TUCKER, C. S., S. W. LLOYD, AND R. L. BUSCH. 1984. Relationship between phytoplankton periodicity and the concentration of total and unionized ammonia in channel catfish ponds. Hydrobiologia 111: 75–79.

VOLLENWEIDER, R. A. 1968. Scientific fundamentals of eutrophication of lakes and flowing waters with particular reference to nitrogen and phosphorus as factors in eutrophication. OECD Report No. DAS CSI 6827, Paris, OECD.

VYHNALEK, V. 1983. Effect of filter-feeding zooplankton on phytoplankton in fish ponds. Int. Rev. Gesamten Hydrobiol. 68: 397–410.

Effects of Manipulations of Aluminum Concentrations and pH on Phosphate Uptake and Photosynthesis of Planktonic Communities in Two Precambrian Shield Lakes

C. Nalewajko and B. Paul[1]

Life Sciences Division, Scarborough Campus, University of Toronto, West Hill, Ont. M1C 1A4

Nalewajko, C., and B. Paul. 1985. Effects of manipulations of aluminum concentrations and pH on phosphate uptake and photosynthesis of planktonic communities in two Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 42: 1946–1953.

From June 1982 to October 1983 we studied the physiological responses of phytoplankton to aluminum perturbations in a circumneutral lake (St. Nora Lake) and an acid-stressed lake (Plastic Lake) on the Precambrian Shield in Ontario. In both lakes, addition of aluminum to water samples decreased microbial phosphate uptake and photosynthesis; significant decreases occurred at 50 $\mu g\ Al \cdot L^{-1}$. Both processes were affected more at pH 5.2–6.9 than at pH 4.5, phosphate uptake was depressed relatively more than photosynthesis, and depression of both processes was larger in Plastic Lake than in St. Nora Lake. Precipitation of phosphate as particles >0.45 μm upon addition of aluminum, as well as direct toxicity of aluminum, appear to be responsible for the observed detrimental effects of aluminum.

Entre juin 1982 et octobre 1983, nous avons étudié les réactions physiologiques du phytoplancton aux variations de la teneur en aluminium dans un lac ayant un pH presque neutre (lac St. Nora) et un lac acide (lac Plastic) situés dans le bouclier précambrien en Ontario. Dans les deux lacs, l'ajout d'aluminium à des échantillons d'eau a entraîné une diminution de l'absorption de phosphate et de la photosynthèse par les microorganismes; il y a eu une baisse marquée lorsque la concentration d'aluminium était de 50 $\mu g \cdot L^{-1}$. Les deux mécanismes ont été touchés davantage lorsque le pH était entre 5,2 et 6,9 que lorsqu'il était à 4,5. La diminution de l'absorption de phosphate a été relativement plus marquée que celle de la photosynthèse et les deux mécanismes ont été touchés davantage dans le lac Plastic que dans le lac St. Nora. La précipitation de phosphate sous forme de particules >0,45 μm après addition d'aluminium de même que la toxicité directe de ce dernier, semblent être les facteurs responsables des effets nocifs observés.

Received July 30, 1984
Accepted August 8, 1985
(J7888)

Reçu le 30 juillet 1984
Accepté le 8 août 1985

Acidic precipitation has been responsible for releasing aluminum from soil into streams and lakes (Johnson et al. 1981; Dickson 1980). In acidic clearwater lakes, aluminum may exceed 650 $\mu g \cdot L^{-1}$, compared with <50 $\mu g \cdot L^{-1}$, in circumneutral lakes (Overrein et al. 1981), and has been implicated as the primary agent in fish toxicity. In comparison with the numerous studies of aluminum toxicity in fish, studies on aquatic primary producers are very few.

As concentrations of aluminum increase in acidic lake water, its chemical speciation may dominate, altering the availability of important plant nutrients. In solutions of $AlCl_3$, for example, phosphate activity was controlled by the total aluminum activity (Hsu and Rennie 1962). In lake water (LaZerte 1984) and in soil, aluminum speciation is very complex, but there is some evidence that one mechanism of aluminum toxicity to higher plants (Foy et al. 1978) and bacteria and fungi (Banik and Dey 1981) may be the decrease of phosphate availability.

Decreased phosphorus availability has also been implicated as the agent responsible for decreasing algal biomass and productivity in acidic aquatic ecosystems (Hendrey et al. 1976). Phosphorus limitation in acidic waters has been demonstrated in enclosure experiments (Wilcox and DeCosta 1982), and may be incurred as a result of the inability of algae to solubilize and utilize phosphates absorbed onto alumino-silicates (Golterman 1973). Jansson (1981) designated aluminum as the agent that induced the production of acid phosphatases by algae in an acidified Swedish lake. Phosphatase production has been used as an indicator of phosphorus limitation (Healey 1975).

Aluminum may also affect phytoplankton in a more direct manner, as a toxin. Although aluminum has been identified as a necessary constituent of some algae (Bertrand and de Wolf 1966), it may be toxic in concentrations as low as 135 $\mu g \cdot L^{-1}$ (Bohm-Tuchy 1959).

Toxicity may be achieved by disrupting ion transport (Clarkson and Sanderson 1971; Upitus et al. 1975). Cellular metabolism may be impaired as a result of intracellular metal–ligand interactions with enzymes (Fluri 1908), adenosine triphosphate (Biessinger and Christensen 1972), and nucleic acids (Mikulski et al. 1982).

Foy and Gerloff (1972) have attempted the only complete study concerning the effects of aluminum on algae, but they

[1]Present address: Biology Department, University of Waterloo, Waterloo, Ont. N2L 3G1.

employed a chelator in the culture medium and did not control pH. As a result, the speciation of the metal during the course of their experiment is uncertain.

The primary intent of this study was to assess short-term responses of phytoplankton photosynthesis and phosphate uptake to an aluminum and pH shock of the magnitude associated with acid precipitation events. Two lakes were selected, one in early stages of acidification and the other circumneutral, to examine any adaptations of the microbiota to the shocks.

Methods

The two lakes that we studied, Plastic (45°11′N, 78°50′W) and St. Nora (45°09′N, 78°50′W), are located on the Precambrian Shield in the Muskoka–Haliburton region of Ontario. Plastic Lake is an oligotrophic, headwater lake with a single basin and is considered to be very sensitive to acidification. The ambient pH ranged from 5.2 to 6.2. St. Nora is larger than Plastic Lake, is mesotrophic, and deemed moderately sensitive to acidification (Ontario Ministry of the Environment 1982). Experiments were carried out at in situ pH's of 6.1–6.9.

At approximately 6-wk intervals, from August 1982 to June 1983, discrete near-surface lake water samples were collected above the deepest part of each lake and transported to the laboratory in a shaded 20-L polycarbonate carboy kept at ambient lake water temperatures.

Final experimental aluminum concentrations in lake water were obtained by 0.1% v/v additions of stock solutions of $Al_2(SO_4)_3 \cdot 16H_2O$. The stock solution pH was 2.2. Immediately following aluminum addition to lake water the pH was adjusted by titration with sodium hydroxide or dilute sulfuric acid. This adjustment was completed in less than 5 min. In several lake water experiments, samples were enriched with 1000 $\mu g\ P \cdot L^{-1}$ as K_2HPO_4.

Pyrex glassware in which the lake water samples were manipulated were soaked in 5% H_2SO_4 for 24 h and rinsed three times in distilled and redistilled water before use. Water alone does not extract aluminum in detectable amounts (Sandell 1959) and in this study was found not to remove aluminum from solution by adsorption onto vessel walls.

Experiments were conducted in an incubator at in situ lake water temperatures. To measure photosynthesis, lake water samples were equilibrated in bottles at a light intensity of 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 1 h after aluminum addition. High specific activity ^{14}C-HCO_3 (1.47 $GBq \cdot mmol^{-1}$; Amersham Corp.) was added to each bottle. Enrichment did not change ambient inorganic carbon concentrations in lake water. Periodically for up to 4 h, a bottle was harvested and two 50-mL aliquots were filtered on 47-mm-diameter cellulose acetate Sartorius filters (0.45-μm porosity). Sample treatment and measurements of radioactivity followed Nalewajko et al. (1981).

Radiophosphorus uptake was measured at 76 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the same time as primary productivity. Carrier-free ^{32}P-PO_4 (New England Nuclear) was added to lake water not more than 30 min after aluminum addition. Isotope incorporation into particulates retained an 0.45-μm-porosity Sartorius membrane filters was monitored periodically for 60–90 min and the radioactivity of the filters measured as in Nalewajko et al. (1981). The rate constant, k (per minute), of radiophosphorus uptake is the curve on a plot of ln percent radioactivity remaining in the filtrate versus time (Lean and Nalewajko 1976). A linear regression program was used to compute k.

Organically complexed aluminum in lake water was measured by passing a 150-mL aliquot through a column containing a cation-exchange resin (Amberlite IR-120H, BDH Chemicals Ltd.) (Driscoll 1984). The difference between aluminum concentrations in the final 100-mL eluate and in the initial lake water sample was considered to be inorganic aluminum.

The exchange column contained 8.0 cm^3 of resin with a total exchange capacity of 15.2 mequiv. The resin was prepared by passing 300 mL of NaCl solution through the column at a rate of 40 $mL \cdot min^{-1}$. Since ionic strength and pH differences between the eluant and sample may alter aluminum speciation, these differences were minimized. The ionic strength of the NaCl solution (0.8129 $mmol \cdot L^{-1}$) was similar to that determined for Plastic Lake (K. Nicholls, Ontario Ministry of the Environment, Box 213, Rexdale, Ont. M9W 5L1). The pH of the eluant, although adjusted to that of the lake water, generally exceeded that of the eluate by 0.5 unit.

Lake water samples were preserved for aluminum analyses in triplicate by addition of dilute sulfuric acid at 1% v/v. After a 10-fold concentration by evaporation, samples were analyzed and aluminum concentrations in stock solutions verified using a Varion atomic absorption spectrophotometer (model AA6) with a nitrous oxide burner head. To suppress interferences caused by ionization of aluminum in the flame, we added potassium as KCl at a final concentration of 2000 $\mu g \cdot mL^{-1}$. The instrument was calibrated periodically with known standards. The limit of detection using this method was 2 $\mu g\ Al \cdot L^{-1}$ in the original water sample.

Results

Abiotic Aluminum Phosphate Interactions

In preliminary experiments, rate constants of radiophosphorus uptake, as determined by radioactivity on 0.45-μm-porosity filters, increased as a linear function of added aluminum concentration. However, appreciable uptake also occurred when ^{32}P-PO_4 and aluminum were added to lake water prefiltered on 0.45-μm-porosity filters and containing no microorganisms (Fig. 1). This abiotic radiophosphorus uptake did not occur if aluminum was not added to filtered lake water. Results on 0.2-μm-porosity polycarbonate filters did not differ significantly (t-test, $P < 0.05$) from the 0.45-μm cellulose acetate filters, indicating that adsorption of dissolved ^{32}P-PO_4, or of dissolved ^{32}P-PO_4 complexed with aluminum, was probably not an important process, and that the retained radioactivity was in particulates exceeding 0.45 μm in size. To calculate biotic radiophosphorus uptake, abiotic uptake measured in prefiltered lake water was subtracted from uptake in whole lake water (Fig. 2).

The prefiltered lake water method we used to estimate abiotic uptake gave data indistinguishable from a procedure in which heat-killed particulates >0.45 μm were introduced at the original concentration to filtered lake water before isotope addition. Similar results were also obtained when carbonyl cyanide-*n*-chlorophenylhydrazone, an uncoupler of photosynthesis, was added to the whole lake water samples (Fig. 2). Therefore the simpler prefiltration method was selected to routinely estimate abiotic radiophosphorus uptake.

Evidence that abiotic uptake involves predominantly the removal of ^{32}P-PO_4 in a particulate form is provided by results from sequential refiltration of filtrates during a ^{32}P-PO_4 uptake experiment (Table 1). With the exception of the data 1 min after isotope addition, all other data show negligible change in radioactivity in filtrate on refiltration. If abiotic uptake consisted of

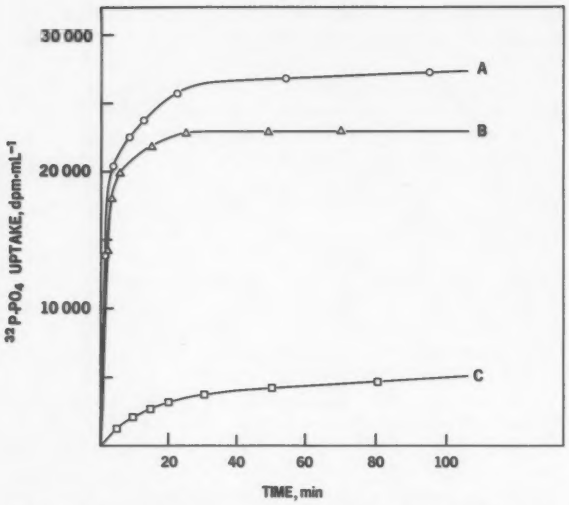


FIG. 1. Radiophosphorus uptake on 0.45-μm-porosity filters. A, whole lake water; B, water prefiltered on 0.45-μm-porosity filters before ^{32}P-PO_4 addition; C, biotic uptake calculated from A−B (St. Nora Lake, 30 September 1982, pH 6.9, added 500 μg $Al \cdot L^{-1}$).

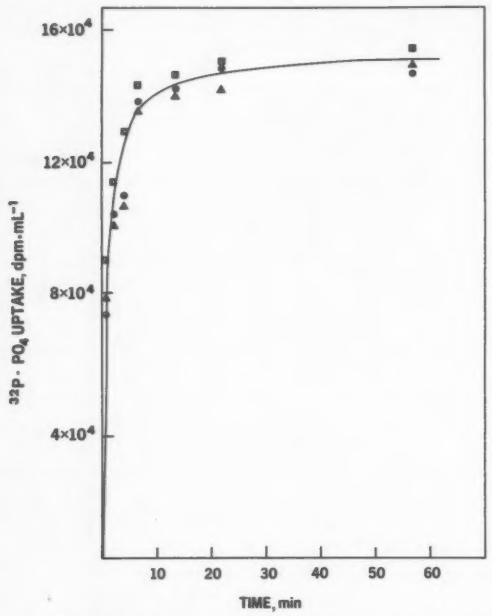


FIG. 2. Radiophosphorus uptake on 0.45-μm-porosity filters from prefiltered lake water (●), lake water plus carbonyl cyanide-*n* chlorophenylhydrazone (▲), and lake water containing heat-killed microorganisms (■) (St. Nora Lake, 28 April 1983, pH 6.1, added 250 μg $Al \cdot L^{-1}$).

dissolved ^{32}P-PO_4 retention by filters, radiophosphorus in the filtrate would have continued to decline on sequential refiltration.

The apparent removal of ^{32}P-PO_4 as a particulate form on the addition of aluminum means that the phosphate pool available for biotic uptake decreases as aluminum concentrations increase. The extent of this decrease at each aluminum concentration was estimated from the asymptote of the appropriate abiotic uptake curve. If, for example, with 500 μg $Al \cdot L^{-1}$, 85% of the radiophosphorus was present in the particulate fraction at the asymptote, the phosphate pool size was deemed to have decreased by this amount. The correction curves in Fig. 3 were derived from a series of abiotic radiophosphorus uptake experiments at 0–100 μg $Al \cdot L^{-1}$ added to filtered lake water, at ambient pH, and at pH 4.5.

TABLE 1. Radioactivity remaining in filtrates during successive refiltrations (1–6) at various times after ^{32}P-PO_4 addition to aluminum-enriched lake water (Plastic Lake, 22 October 1983). Samples were refiltered using new filters at 15-s intervals. Total activity added at time 0 was 20 500 dpm $\cdot$ mL^{-1}.

Time after isotope addition (min)	Filtrate radioactivity (dpm·mL^{-1})					
	1	2	3	4	5	6
1	15 023	4622	4473	4458	4409	4384
8	2 998	2488	2091	2023	1990	1954
15	2 653	2021	1986	1954	1908	1891
24	3 456	3030	2886	2812	2728	2706
33	3 125	2475	2420	2376	2344	2330
40	3 442	2579	2488	2435	2403	2388

In all experiments involving aluminum addition the biotic ^{32}P-PO_4 uptake rate constants were normalized to the phosphate pool size of the control. For example, at in situ pH, on addition of 200 μg $Al \cdot L^{-1}$, 30% of the ^{32}P-PO_4 was removed abiotically in the particulate fraction (Fig. 4). Therefore the rate constant would be multiplied by 100/70; for 600 μg $Al \cdot L^{-1}$ the factor would be 100/22. Precipitation of radiophosphate on aluminum addition to lake water adjusted to pH 4.5 (Fig. 3) was less dramatic than at ambient pH.

Biological Effects of Aluminum

Radiophosphorus uptake experiments at ambient pH and at a range of added aluminum concentrations were carried out on six occasions during 28 July 1982 to 22 October 1983. At the same time, six experiments were carried out in Plastic Lake and three in St. Nora Lake, respectively, using samples of lake water adjusted to pH 4.5. In the absence of clear seasonal trends in the effect of added aluminum on radiophosphate uptake, rate constants, as percentages of the control, for each added aluminum concentration were pooled (Fig. 4). Ambient aluminum concentrations in the two lakes were <66 μg $\cdot L^{-1}$ (Table 2).

In both lakes, aluminum significantly depressed phosphate uptake at in situ pH. Since these data have been corrected for abiotic uptake and for the effects of aluminum on phosphate pool size, the depression may be interpreted as a toxic, biological effect. At pH 4.5 (Fig. 4) the effects of aluminum were less marked, but the data base was smaller and variability higher than at ambient lake water pH.

Effects of aluminum addition on photosynthesis were investigated at the time of the radiophosphorus experiments. To allow comparisons of data generated throughout the study period, photosynthetic rates were normalized to percentages of the relative control. Since seasonal trends in amounts of depression by a given aluminum concentration were not discernible, means were calculated for each added aluminum concentration, as in the radiophosphorus experiments.

In experiments at in situ pH, photosynthesis exhibited a

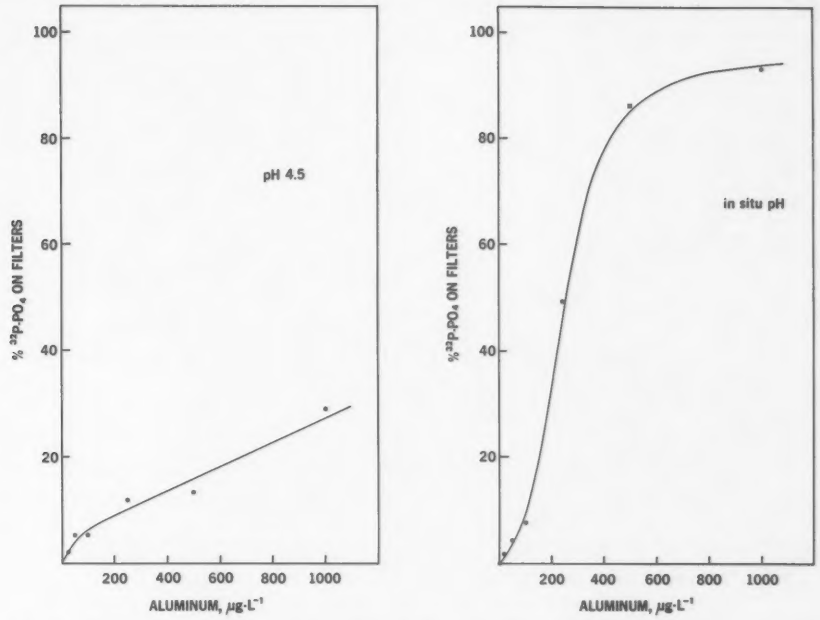


FIG. 3. Percentage of ^{32}P-PO$_4$ retained from 0.45-μm prefiltered lake water on 0.45-μm-porosity filters, at the asymptote, as a function of added aluminum concentrations.

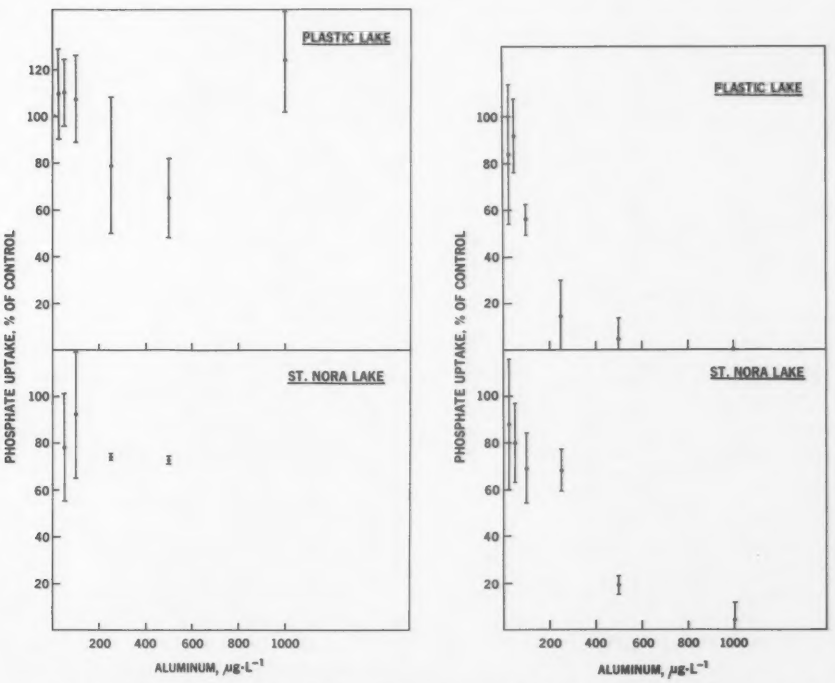


FIG. 4. Mean phosphate uptake rates for experiments conducted at various added aluminum concentrations at pH 4.5 (left) and at in situ pH (right) in Plastic and St. Nora lakes. Error bars represent standard deviations.

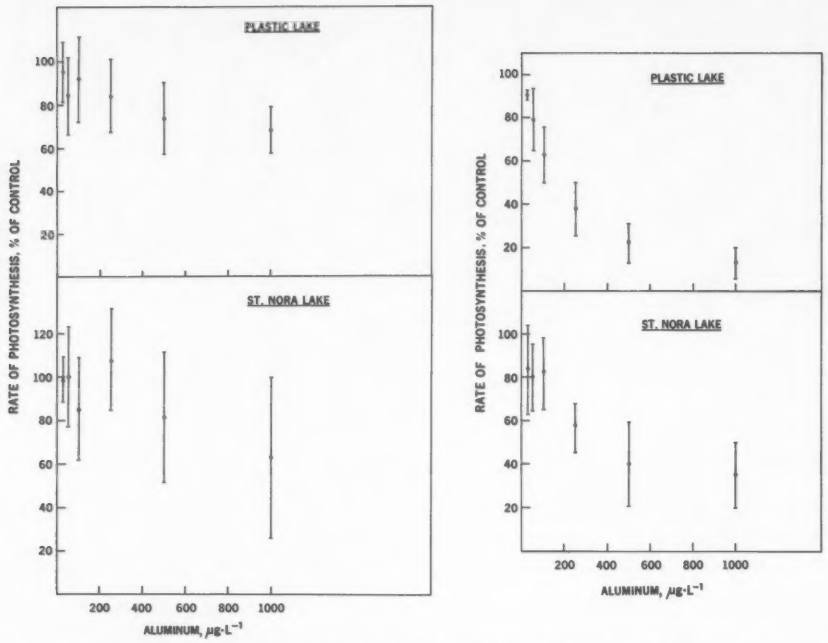


FIG. 5. Mean photosynthetic rates for experiments conducted at various added aluminum concentrations at pH 4.5 (left) and at in situ pH (right) in St. Nora and Plastic lakes. Error bars represent standard deviations.

TABLE 2. Temporal changes in organically complexed and inorganic aluminum in Plastic and St. Nora lakes (Oct. 1982 – Sept. 1983).

Lake	Date	Aluminum concentration ($\mu g \cdot L^{-1}$) Organic	Inorganic	Total
Plastic	21 Oct. 1982	36	30	66
Plastic	18 Nov. 1982	13	53	66
Plastic	21 Jan. 1983	36	9	45
Plastic	17 Mar. 1983	30	34	64
Plastic	31 Mar. 1983	36	12	48
Plastic	28 Apr. 1983	54	6	60
Plastic	22 Oct. 1983	45	9	54
St. Nora	30 Sept. 1982	56	0	56
St. Nora	16 Oct. 1982	44	0	46
St. Nora	25 Nov. 1982	43	0	42
St. Nora	21 Jan. 1983	50	0	52
St. Nora	17 Mar. 1983	38	0	40
St. Nora	31 Mar. 1983	38	0	40
St. Nora	29 Apr. 1983	40	0	40
St. Nora	31 May 1983	40	0	40

graded response to aluminum additions (Fig. 5). A less marked trend of depression occurred at pH 4.5, and variability in the data was high due to a small data base.

Photosynthesis was similar in lake water samples to which aluminum was added as sulfate or nitrate salts (t-test, $P < 0.05$), indicating that the added cation was the agent causing the observed depression.

The effect of phosphate enrichment on the response of photosynthetic rates to added aluminum was investigated in both lakes (Table 3). Enrichment with 1000 $\mu g\ P \cdot L^{-1}$ had no

TABLE 3. Photosynthetic rates ($\mu g\ C \cdot L^{-1} \cdot h^{-1}$) in experiments with added aluminum and phosphorus in Plastic (23 Jan. 1983) and St. Nora (16 Oct. 1983) lakes (ND = not determined).

	Plastic Lake	St. Nora Lake
Control	0.060	3.24
50 $\mu g\ Al \cdot L^{-1}$	0.049	ND
250 $\mu g\ Al \cdot L^{-1}$	0.018	2.24
1000 $\mu g\ P \cdot L^{-1}$	0.060	3.46
50 $\mu g\ Al \cdot L^{-1}$ + 1000 $\mu g\ P \cdot L^{-1}$	0.056	ND
250 $\mu g\ Al \cdot L^{-1}$ + 1000 $\mu g\ P \cdot L^{-1}$	0.044	2.95

effect on photosynthesis in Plastic Lake but stimulated it somewhat in St. Nora Lake. Addition of 250 $\mu g\ Al \cdot L^{-1}$ decreased photosynthesis to 30 and 60%, respectively, of the control. When the aluminum and phosphate were added simultaneously, photosynthesis was decreased only to 73.3 and 91%, of the control, respectively, in Plastic and St. Nora Lakes.

Phosphate uptake and photosynthesis as a function of aluminum concentration were positively correlated (Fig. 6, $P < 0.001$), but photosynthesis still proceeded at 23% of control when phosphate uptake was zero.

To determine if the observed depression of photosynthesis and phosphate uptake was dependent on the duration of exposure to aluminum, experiments were conducted using St. Nora Lake water after various equilibration times with aluminum (Fig. 7). Responses to aluminum were elicited immediately upon addition, but stable depressions of phosphate uptake and photosynthesis were achieved after 15 min and 7 h, respectively.

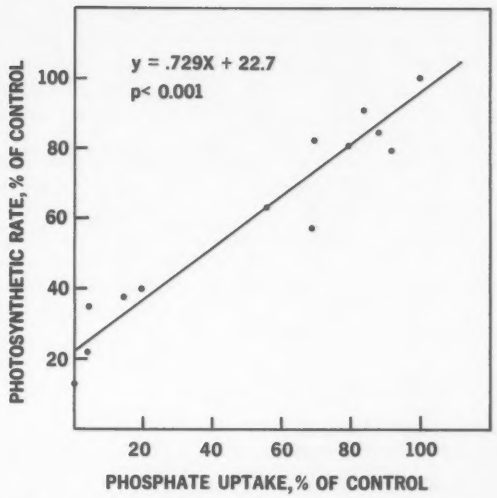


FIG. 6. Mean photosynthetic rates as a function of phosphate uptake rates in Plastic and St. Nora lakes for each added aluminum concentration.

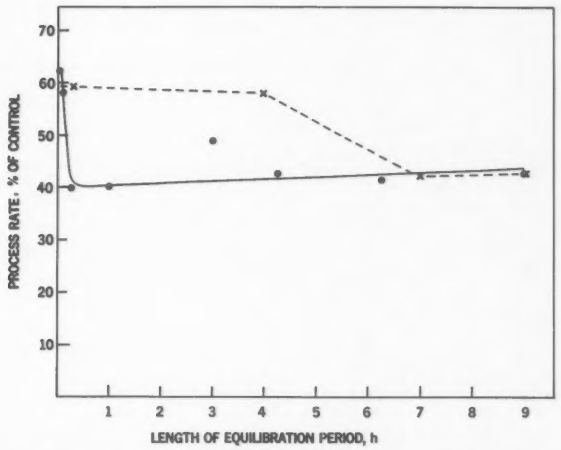


FIG. 7. Phosphate uptake (●) and photosynthetic rates (×) as a function of length of equilibrium period with aluminum before isotope addition (St. Nora Lake, 29 April 1982, pH 6.1, added 250 μg $Al \cdot L^{-1}$).

Discussion

Our data indicate that removal of phosphate from solution as particulates >0.45 μm is likely to occur when aluminum concentrations increase in lake water, particularly at pH 6. As a result the phosphate pool size available for microbial uptake decreases. Further, the aluminum appears to decrease phosphate uptake through direct toxicity to the microbiota, and depresses phytoplankton photosynthesis. Only a portion of depression is related to decreased phosphorus availability, as indicated by only partial reversal on enrichment with phosphate.

Significant depressions of photosynthesis and phosphate uptake were observed at aluminum concentrations characteristic of acidic lakes. Aluminum caused greater relative inhibition of photosynthesis and phosphate uptake at in situ pH's of 5.2–6.9 than at pH 4.5, although the absolute rates of these processes at pH 4.5 were substantially lower. These results support the conclusions of Driscoll et al. (1980) that, as in their experiments with fish, aluminum hydroxide complexes, which would predominate at higher pH, are the more toxic species. As a result the effects of aluminum in circumneutral lakes may be of greater importance than in acidic waters. However, the free aluminum aquo ion appears to be more toxic than complexed aluminum forms to fungi (Firestone et al. 1983) and higher plants (Magistad 1926). It is also possible that increased dissolved phosphate concentrations observed in Plastic Lake and St. Nora Lake on acidifying samples from ambient pH to pH 4.4–5 (C. Nalewajko and M. O'Mahoney, unpubl. data) decrease the relative phosphorus stress imposed on the microorganisms when aluminum is added at the lower pH.

The depression of phosphate uptake and photosynthesis by added aluminum at ambient lake water pH was more pronounced in Plastic Lake than in St. Nora Lake. One explanation is that particulate aluminum formation would be more extensive at the neutral pH in St. Nora Lake, thereby decreasing dissolved aluminum concentrations below those in Plastic Lake when identical aluminum enrichments are made in our experiments. Differences in the proportions of organic and inorganic aluminum forms could also be invoked. Our data (Table 2) show aluminum in St. Nora Lake to be present exclusively in the organic form documented to be the less toxic, at least to brook trout (*Salvelinus fontinalis*) (Baker and Schofield 1982), but the ion exchange method we used to separate inorganic from organic forms is now known to be subject to errors (C. Driscoll, Syracuse University, Syracuse, NY 13210, pers. comm.). Although total aluminum concentrations in the two lakes are not very different (Table 2), aluminum speciation is no doubt different, and the resulting complexes may elicit different responses from the biota (Driscoll et al. 1980; Firestone et al. 1983). Finally, differential susceptibility may be due to differences in phytoplankton species composition between the two lakes (Paul 1984; O'Mahoney 1985). A number of authors have detected differential species or strain susceptibility to metal toxicity (Stokes 1975), and differences in the ability to use phosphates complexed with aluminum may also be involved (Golterman 1973; Taylor et al. 1960).

Both phosphate uptake and photosynthesis show an immediate depression following aluminum addition, but there is a difference in the time scale of the next phase, in which process rates decline further, to a stable level (Fig. 7). The initial rapid depression of phosphate uptake may be due to a sudden decrease of phosphate concentration, but the same explanation is not likely for photosynthesis. Abiotic ^{14}C uptake, or large *y*-intercepts, were not observed in experiments on the time course of photosynthesis (Paul 1984), which indicates that the dissolved inorganic carbon concentration in the medium did not change following aluminum addition. In view of this a mechanism not based on pool size changes must be invoked. Possibly, aluminum alters the plasmalemma surface charge, which would disrupt ion transport (Clarkson and Sanderson (1971)) immediately and nonspecifically. Bohm-Tuchy (1959) and Hofler (1959) reported that aluminum may render the algal plasmalemma impermeable to ion transport.

Phosphate uptake appears more susceptible than photosynthesis to inhibition by aluminum. Not only is the stable depression of phosphate uptake achieved much faster, but complete depression occurs at concentrations of aluminum which permit photosynthesis to proceed at about 23% of the

control. We can only speculate that abiotic precipitation of phosphorus by aluminum inside the cell could result in this greater sensitivity of phosphate uptake relative to photosynthesis even if enzymes and other molecules involved in the two processes were not differentially sensitive to aluminum.

The prevention of substantial depression of photosynthesis by adding phosphate simultaneously with aluminum indicates that the control by aluminum of phosphate availability may be an important mechanism controlling primary production in lakes. Incomplete restoration of photosynthesis rates may be attributed to toxicity of residual aluminum not precipitated with phosphate. The populations were not very phosphorus deficient, as indicated by the absence of substantial stimulation when phosphate was added alone; therefore, it is not likely that the incomplete restoration of photosynthesis by phosphorus plus aluminum addition was caused by incomplete reversal of phosphorus deficiency. In addition to removing aluminum by precipitation, phosphate may decrease the toxic action of the metal through other complexation reactions (Hartwell and Pember 1918).

Although our experiments demonstrate that precipitation of phosphate can be substantial when aluminum is added at concentrations existing in many acidic lakes, the experimental design may be challenged as not truly representative of real events in lakes. It is, therefore, reassuring that coprecipitation is supported by data from Swedish lakes, in which phosphate and aluminum concentrations are negatively correlated (Overrein et al. 1981; Dickson 1980).

Aluminum phosphate and aluminum hydroxide solubilities are controlled by solution pH (Stumm and Morgan 1981). Consequently, aluminum phosphate interactions are also pH dependent (Hsu and Jackson 1960). In this investigation, maximum binding occurred at pH 6.0, the thermodynamically predicted value for minimum aluminum phosphate and hydroxide solubilities (Stumm and Morgan 1981). Complexation decreased as the pH was raised and lowered from 6.0. Dickson (1978) reported similar results from an acidic lake.

Aluminum precipitates have been observed in stream beds in the central and western United States and in Sweden where acid waters with elevated aluminum concentrations mixed with stream waters of higher pH (Theobald et al. 1963; Herrmann and Baron 1980; Hultberg and Johannson 1981). Theobald et al. (1963) reported the pH of the zone of maximal deposition in these waterways to be 6.0.

Aluminum enters lakes predominantly as a component of acidic runoff following precipitation events or spring snowmelt (Driscoll 1980; LaZerte 1984). According to our results, as neutralization processes in the lake proceed, interactions between the aluminum and phosphate would intensify, thus accelerating the oligotrophication of acidifying lakes. Evidence of this hypothesis comes from observations of decreased aluminum (Driscoll et al. 1982; Dillon et al. 1979) and phosphate concentrations (Hultberg and Andersson 1982; Adamski and Michalski 1975) in water columns immediately following neutralization procedures. Similarly, aluminum additions to enclosures in a shallow eutrophic lake are accompanied by decreases in pH from 8.7 to 6.0, and decreased phosphate and chlorophyll concentrations (Zarini et al. 1983). As pH's are altered and approach those determined for maximal aluminum phosphate binding, phytoplankton biomass is decreased (Scheider and Dillon 1976; Yan and Stokes 1976). Dillon et al. (1979) reported immediate increases in phytoplankton biomass only when fertilization with phosphate accompanied neutralization endeavours. Similarly, as indicated by microcosm experiments, algal productivity may be limited by coincidental decreases of aluminum and phosphorus levels during the recovery process of strip mine lakes (King et al. 1974). Further, the predominant phosphate forms in acidified sediments are aluminum and iron phosphates (Nur and Bates 1979).

Our data confirm the importance of chemical interactions between aluminum and phosphate, and we suggest that available phosphate in lake water would decline quickly following an increase in aluminum concentrations, particularly at a pH close to 6. The aluminum also has a direct negative effect on at least two important microbial processes, phosphate uptake and photosynthesis, which decrease drastically at aluminum concentrations in the range encountered in acidic lakes.

Acknowledgments

We thank C. Manbeck, S. Mendel, and M. O'Mahony for their assistance and Drs. D. Voltolina, C. Driscoll, and B. LaZerte for constructive criticism of the manuscript. This research was supported by grants to C. Nalewajko from the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of the Environment.

References

ADAMSKI, J. M., AND M. F. P. MICHALSKI. 1975. Reclamation of acidified lakes — Middle and Lhi, Sudbury, Ontario. Verh. Int. Ver. Limnol. 19: 1971–1983.

BAKER, J. P., AND C. L. SCHOFIELD. 1982. Aluminum toxicity to fish in acidic waters. Water Air Soil Pollut. 18: 289–309.

BANIK, S., AND B. K. DEY. 1981. Alluvial soil microorganisms capable of utilizing insoluble aluminum phosphate as a sole source of phosphorus. Zbl. Mikrobiol. 138: 437–442.

BERTRAND, D., AND A. DE WOLF. 1966. L'aluminum oligo-element dynamique pour les vegetaux superieur. C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat. 266: 479–481.

BIESSINGER, K. E., AND G. M. CHRISTENSEN. 1972. Effects of various metals on survival growth, reproduction and metabolism of *Daphnia magna*. J. Fish. Res. Board Can. 29: 1691–1700.

BOHM-TUCHY, E. 1959. Plasmalemma und Aluminiumsalz-Wirkung. Protoplasma 52: 108–142.

CLARKSON, D. T., AND J. SANDERSON. 1971. Inhibition of the uptake and long distance transport of calcium by aluminum and other polyvalent cations. J. Exp. Bot. 22: 837–851.

DICKSON, W. 1978. Some effects of the acidification of Swedish lakes. Verh. Int. Ver. Limnol. 20: 851–856.

1980. Properties of acidified waters, p. 75–83. *In* D. Drablos and A. Tollan [ed.] Proc. Int. Conf. Ecol. Impact of Acid Precip., SNSF Project, Oslo, Norway.

DILLON, P. J., N. D. YAN, W. A. SCHEIDER, AND N. CONROY. 1979. Acidic lakes in Ontario, Canada: characterization, extent and responses to base and nutrient additions. Arch. Hydrobiol. Beih. 13: 317–336.

DRISCOLL, C. T. 1980. Chemical characterization of some dilute acidified lakes and streams in the Adirondack region of New York State. Ph.D. thesis, Cornell University, Ithaca, NY. 309 p.

1984. A procedure for the fractionation of aqueous aluminum in dilute acidic waters. Int. J. Environ. Anal. Chem. 16: 267–283.

DRISCOLL, C. T., J. P. BAKER, J. J. BISOGNI, AND C. L. SCHOFIELD. 1980. Effect of aluminum speciation on fish in dilute acidified waters. Nature (Lond.) 284: 161–164.

DRISCOLL, C. T., J. R. WHITE, G. C. SCHAFRAN, AND J. D. RENDALL. 1982. $CaCO_3$ neutralization of acidified surface waters. J. Environ. Eng. Div. 108: 1128–1146.

FIRESTONE, M. K., K. KILLHAM, AND J. G. MCCOLL. 1983. Fungal toxicity of mobilized soil aluminum and manganese. Appl. Environ. Microbiol. 46: 758–761.

FLURI, M. 1908. The influence of aluminum salts on protoplasm. Flora 99: 81–126.

FOY, C. D., R. L. CHANEY, AND M. C. WHITE. 1978. The physiology of metal toxicity in plants. Annu. Rev. Plant Physiol. 29: 511–566.

FOY, C. D., AND G. C. GERLOFF. 1972. Response of *Chlorella pyrenoidosa* to aluminum and low pH. J. Phycol. 8: 268–271.

GOLTERMAN, H. L. 1973. Natural phosphate sources in relation to phosphate budgets: a contribution to the understanding of eutrophication. Water Res. 7: 3–17.

HARTWELL, B. L., AND F. R. PEMBER. 1918. The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. Soil Sci. 6: 259–280.

HEALEY, F. P. 1975. Physiological indicators of nutrient deficiency in algae. Fish. Mar. Serv. Res. Dev. Tech. Rep. 585: 30 p.

HENDREY, G. R., K. BAALSRUD, T. S. TRAAEN, M. LAAKE, AND G. RADDUM. 1976. Acid precipitation: some hydrobiological changes. Ambio 5: 224–227.

HERRMANN, R., AND J. BARON. 1980. Aluminum mobilization in acid stream environments, Great Smokey Mountains National Park, U.S.A., p. 218–219. *In* D. Drablos and A. Tollan [ed.] Proc. Int. Conf. Ecol. Impact of Acid Precip., SNSF Project, Oslo, Norway.

HOFLER, K. 1959. Aluminiumsalz-Wirkung auf Spirogyren und Zygnemem. Protoplasma 50: 248–258.

HSU, P. H., AND M. L. JACKSON. 1960. Inorganic phosphate transformations by chemical weathering in soils as influenced by pH. Soil Sci. 90: 16–24.

HSU, P. H., AND D. A. RENNIE. 1962. Reactions of phosphate in aluminum systems. I. Adsorption of phosphate by X-ray amorphous "aluminum hydroxide." Can. J. Soil Sci. 42: 197–209.

HULTBERG, H., AND I. D. ANDERSON. 1982. Liming of acidified lakes: induced long-term changes. Water Air Soil Pollut. 18: 311–337.

HULTBERG, H., AND S. JOHANNSON. 1981. Acid groundwater. Nord. Hydrol. 12: 51–64.

JANSSON, M. 1981. Induction of high phosphatase activity by aluminum in acid lakes. Arch. Hydrobiol. 93: 32–44.

JOHNSON, N. H., C. T. DRISCOLL, J. S. EATON, G. E. LIKENS, AND W. H. MCDOWELL. 1981. 'Acid rain,' dissolved aluminum and chemical weatherings at the Hubbard Brook Experimental Forest, New Hampshire. Geochim. Cosmochim. Acta 45: 1421–1437.

KING, D. L., J. L. SIMMER, C. S. DECKAR, AND C. W. OGG. 1974. Acid strip mine lake recovery. J. Water Pollut. Control Fed. 46: 2301–2315.

LAZERTE, B. D. 1984. Forms of aqueous aluminum in acidified catchments of central Ontario: a methodological analysis. Can. J. Fish. Aquat. Sci. 41: 766–776.

LEAN, D. R. S., AND C. NALEWAJKO. 1976. Phosphate exchange and organic phosphorous excretion by freshwater algae. J. Fish. Res. Board Can. 33: 1312–1323.

MAGISTAD, O. C. 1926. The aluminum content of the soil solution and its relation to soil reaction and plant growth. Soil Sci. 20: 181–224.

MIKULSKI, C. M., S. COCCO, L. MATTUCCI, N. DEFRANCO, AND L. WEISS. 1982. Aluminum chloride complexes with purine, adenine and guanine. Inorg. Chim. Acta 67: 173–175.

NALEWAJKO, C., K. LEE, AND H. SHEAR. 1981. Phosphorus kinetics in Lake Superior: light intensity and phosphate uptake in algae. Can. J. Fish. Aquat. Sci. 38: 224–232.

NUR, R., AND M. H. BATES. 1979. The effects of pH on the aluminum, iron and calcium phosphate fractions of lake sediments. Water Res. 13: 813–815.

O'MAHONEY, M. A. 1985. A seasonal study of an acidifying and circumneutral shield lake: effect of pH shock on phosphorus concentrations and kinetics, and photosynthesis. M.Sc. thesis, University of Toronto, Toronto, Ont. 188 p.

ONTARIO MINISTRY OF THE ENVIRONMENT. 1982. Acid sensitivity survey of lakes in Ontario. Ont. Minist. Environ. APIOS Rep. No. 003/82, Rexdale, Ont.

OVERREIN, L. N., H. M. SEIP, AND A. TOLLAN. 1981. Acid precipitation — effects on forest and fish. Final Report of the SNSF project 1972–1980. 175 p.

PAUL, B. J. 1984. The effect of aluminum on phytoplankton. M.Sc. thesis, University of Toronto, Toronto, Ont. 196 p.

SANDELL, E. B. 1959. Calorimetric determination of traces of metals. 3rd ed. Interscience. New York, NY. 456 p.

SCHEIDER, W. A., AND P. J. DILLON. 1976. Neutralization and fertilization of acidified lakes near Sudbury, Ontario, p. 93–100. *In* P. H. Jones [ed.] Proc. 11th Can. Symp. Water Pollut. Res. Canada Council of Inland Waters, Burlington, Ont.

STOKES, P. 1975. Uptake and accumulation of copper and nickel by metal-tolerant strains of *Scenedesmus*. Verh. Int. Ver. Limnol. 19: 2128–2137.

STUMM, W., AND J. J. MORGAN. 1981. Aquatic chemistry. An introduction emphasizing chemical equilibria in natural waters. John Wiley & Sons, New York, NY. 583 p.

TAYLOR, A. W., E. L. GURNEY, AND W. L. LINDSAY. 1960. An evaluation of some iron and aluminum phosphates as sources of phosphate for plants. Soil Sci. 90: 25–31.

THEOBALD, P. K., H. W. LAKIN, AND D. B. HAWKINS. 1963. The precipitation of aluminum, iron, and manganese at the junction of Deer Creek with Snake River in Summit County, Colorado. Geochim. Cosmochim. Acta. 27: 121–132.

UPITIS, V., A. NOLLENDORFA, AND D. PAKALNE. 1975. Little-studied trace elements in *Chlorella* culture. Aluminum. Latv. PSR Zinat. Akad. Vestis. 3: 21–28.

WILCOX, G., AND J. DECOSTA. 1982. The effect of phosphorus and nitrogen addition on the algal biomass and species composition of an acidic lake. Arch. Hydrobiol. 94: 393–424.

YAN, N. D., AND P. M. STOKES. 1976. The effects of pH on lakewater chemistry and phtoplankton in a La Cloche Mountain lake, p. 127–136. *In* P. H. Jones [ed.] Proc. 11th Can. Symp. Water Pollut. Res. Canada Council of Inland Waters, Burlington, Ont.

ZARINI, S., D. ANNONI, AND O. RAVERA. 1983. Effects produced by aluminum in freshwater communities studied by the "enclosure" method. Environ. Technol. Lett. 4: 247–256.

Fertilization and Egg Retention in Artificially Inseminated Female American Lobsters, *Homarus americanus*

S. L. Waddy and D. E. Aiken

Department of Fisheries and Oceans, Fisheries and Environmental Sciences, Fisheries Research Branch, Biological Station, St. Andrews, N.B. E0G 2X0

Waddy, S. L., and D. E. Aiken. 1985. Fertilization and egg retention in artificially inseminated female American lobsters, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 42: 1954–1956.

Female American lobsters, *Homarus americanus*, seldom are receptive to mating more than 24 h after they molt, a fact that complicates breeding programs in large facilities where animals are held individually. Artificial insemination can eliminate this problem, but preliminary studies have indicated poor fertilization success from intermolt inseminations. In the present study, 86 female American lobsters of wild and cultured stock were artificially inseminated in all postmolt and intermolt stages, 64 subsequently spawned, and 98% successfully fertilized their eggs, indicating no constraint of molt stage on success of insemination. Of artificially inseminated females, 50% of wild stock and 7% of cultured stock carried their eggs to hatch.

Les homards américains (*Homarus americanus*) femelles sont rarement disposés à s'accoupler plus de 24 h après avoir mué, ce qui complique les programmes de reproduction dans les grosses installations où l'on garde les animaux séparés. L'insémination artificielle peut éliminer ce problème, mais des études préliminaires ont montré que l'insémination faite entre les mues ne donne qu'un faible taux de fécondation. Dans le cadre de la présente étude, on a procédé à l'insémination artificielle de 86 homards américains femelles provenant d'un stock sauvage et d'un stock d'élevage, à toutes les étapes qui suivent la mue et entre les mues; par la suite, 64 ont libéré leurs œufs et, dans 98 % des cas, ceux-ci étaient fécondés, ce qui indique que l'étape de la mue n'entrave pas le succès de l'insémination. Parmi les femelles inséminées artificiellement, 50 % de celles qui provenaient du stock sauvage et 7 % de celles qui provenaient du stock d'élevage ont porté leurs œufs jusqu'à éclosion.

Received April 17, 1985
Accepted August 8, 1985
(J8203)

Reçu le 17 avril 1985
Accepté le 8 août 1985

Female American lobsters, *Homarus americanus*, seldom are receptive to mating more than 24 h after ecdysis (Aiken and Waddy 1980 for review). In nature or in communal culture tanks this is seldom a problem, but where valuable broodstock animals are held individually, the unobserved molt of a valuable female can mean the loss of a year or two of egg production (Waddy and Aiken 1984).

Artificial insemination — the manual placement of a male spermatophore in the female seminal receptacle — could overcome this problem. If females could be artificially inseminated whenever convenient between molts, the necessity for human presence at the time of the female molt would be eliminated.

Fertile eggs have been obtained from American lobsters that were artificially inseminated immediately after ecdysis (Talbot 1984), but our preliminary attempts to obtain fertile eggs from hardshell intermolt inseminations were not encouraging (Aiken et al. 1984). However, male and hardshell intermolt female lobsters occasionally mate in the laboratory (Aiken and Waddy 1980; Dunham and Skinner-Jacobs 1978), and although this is uncommon, it indicates that insemination need not be restricted to the postmolt (stage A) period.

Because the potential of artificial insemination will not be realized unless eggs can be fertilized from inseminations performed at any time between molts, our objective was to determine the relationship between molt stage at insemination and the fertility of eggs subsequently extruded.

Methods

Eighty-six mature female American lobsters were artificially inseminated between July 1983 and July 1984, using the technique described in Aiken et al. (1984). Females were either wild ($N = 8$), obtained from the southern Gulf of St. Lawrence, or cultured stock ($N = 56$), hatched and reared at 20°C. All animals in the study were isolated from males and held individually under local photoperiod and temperature in standard broodstock tanks (Aiken and Waddy 1985). Inseminations were done between postmolt day 0 and day 341. Molt stages at insemination were determined by shell hardness (Aiken 1980). Of the 64 that spawned, 20 were in stage A, 12 in stages B–C_1, 13 in stages C_2–C_3, and 19 in stage C_4. Eggs were microscopically examined 4–5 d after spawning for cleavage planes (Bumpus 1891), which are found only in fertilized eggs. Ovigerous females were examined monthly during the incubation period and the proportion of attached eggs was estimated using a method similar to that described by Talbot et al. (1984).

Results

Of the 86 female lobsters artificially inseminated in all postmolt and intermolt stages from A to C_4, 64 spawned during the study and 98% fertilized their eggs (Table 1). The one female carrying unfertilized eggs was inseminated only 4 d prior to spawning. All others were inseminated at least 17 d before

TABLE 1. Relationship between female lobster (*H. americanus*) molt stage at time of artificial insemination and incidence of fertilization.

Female molt stage at insemination	A	B–C_1	C_2–C_3	C_4
N (cultured)	18	10	11	17
N (wild)	2	2	2	2
Molt–insemination interval (days)	0–1	2–14	16–90	109–341
Insemination–spawning interval (days)	250–368	29–358	25–361	4–267
% of females with fertilized eggs	100	100	100	95

TABLE 2. Correlation between female lobster (*H. americanus*) history and retention of eggs after artificial insemination.

	Female history	
	Cultured (reared at 20°C)	Wild (Gulf of St. Lawrence)
Number of females retaining eggs to eyed stage	41/56 (73%)	8/8 (100%)
Average estimate of eggs present at eyed stage	22%	43%
Number of females retaining eggs to hatch	4/56 (7%)	4/8 (50%)
Average estimate of eggs present at hatch	25%	63%
Number of females without detectable egg loss at hatch	1 (2%)	2 (25%)

spawning. Most females (77%) retained some eggs to the eyed stage (6–9 wk after spawning at our water temperatures). All females that lost their attached eggs prior to appearance of eye pigment were of cultured stock (Table 2). Numbers of females retaining eggs to later stages of development decreased with time, and at hatch, only 50% of wild females and 7% of cultured females were still carrying eggs. No relationship could be established between molt stage at insemination and degree of egg loss. The only significant difference in quantity of eggs retained is that more females of wild stock (50%) than of cultured stock (7%) retained eggs throughout the study (Table 2). Individual females of wild stock also retained a greater proportion of their eggs, an average of 63% compared with only 25% for cultured stock. Two wild (25%) and one cultured female (2%) hatched without detectable egg loss (Table 2) and these females were inseminated in stages A, C_1, and C_4. Larval survival from these artificially inseminated females was equivalent to that in naturally mated lobsters.

Discussion

Before an extruded egg can hatch successfully it must be fertilized, it must attach securely to the nonplumose setae on the ventral abdomen, and it must remain attached until embryonic development is complete. This can take from 4 to 12 mo depending on temperature.

Fertilization is essential to the attachment process. Unfertilized eggs either fail to attach or they attach and drop off over succeeding days or weeks. However, lack of fertilization is not the only reason eggs are lost. Not all of the causes are known, but poor water quality, unfavorable environmental conditions, poor handling practices, and presence of macro- and microbial epibionts have been implicated (Aiken and Waddy 1980, 1985; Aiken et al. 1985; Harper and Talbot 1984; Talbot 1984; Talbot and Harper 1984; Talbot et al. 1984).

From our preliminary work, we (Aiken et al. 1984) reported that successful fertilization occasionally resulted from artificial inseminations in molt stage C_4 but not from insemination in earlier molt stages. The present study, which shows that fertilization can occur successfully from inseminations during all preovigerous molt stages, appears to conflict with those preliminary results. However, the conflict is a result of differing methodology. In the earlier study we used only cultured females, and our criterion for successful fertilization was the appearance of eye pigment on the embryo. Cultured females are much more prone to lose their eggs, even if properly fertilized. Since the "eyed stage" does not appear until 6–9 wk after fertilization, fertilized eggs that were lost in the first 2 mo were improperly recorded as unfertilized. This problem was eliminated here by examining the extruded eggs for cleavage planes indicative of embryonic development and therefore fertilization.

Egg loss has been a problem for groups attempting to breed lobsters and develop cultured stocks. We have had exceptional success obtaining egg attachment and retention through embryonic development to hatching, using wild broodstock maintained under natural photoperiod and temperature cycles. In contrast, wild females held in coastal water temperatures of central California rarely carry their eggs to hatching without significant loss (Conklin 1983; Hedgecock 1983; Talbot and Harper 1984; Talbot et al. 1984). This suggests that local environmental conditions may be important in egg retention (Aiken and Waddy 1985).

Although we find no difference in incubation success of females naturally mated in the wild and those of wild stock naturally mated in our laboratory, fewer than half of our naturally mated cultured females (hatched and reared to maturity at 20°C) carry a full component of eggs to hatching, and the situation appears to be even worse with cultured females held in California (Talbot and Harper 1984; Talbot et al. 1984). It is therefore important that artificially inseminated wild females in this study had much higher egg retention rates than artificially inseminated cultured females. Wild females would seem to be a better assay of the potential of artificial insemination.

Here we eliminated infertility as a possible cause of the egg loss we observed previously. The eggs of artificially inseminated females were fertile, irrespective of the molt stage at time of insemination. This is important because the value of artificial insemination would be compromised unless it gave the culturist considerable latitude concerning time of insemination. The necessity of identifying a specific point in molt stage C_4 (as was indicated by our preliminary study) would be scant improvement over the current requirement that the culturist be present at the time of ecdysis in order to arrange a natural mating. The data we report here show that insemination at any preovigerous molt stage can result in fertile eggs at extrusion, and that artificial insemination therefore has considerable potential in lobster breeding programs. The problem now is not how to fertilize the eggs, but how to ensure their attachment and retention throughout the incubation period.

References

AIKEN, D. E. 1980. Molting and growth, p. 91–163. *In* J. S. Cobb and B. F. Phillips [ed.] The biology and management of lobsters. Vol. 1. Academic Press, New York, NY.

AIKEN, D. E., AND S. L. WADDY. 1980. Reproductive biology, p. 215–276. *In* J. S. Cobb and B. F. Phillips [ed.] The biology and management of lobsters. Vol. 1. Academic Press, New York, NY.

1985. Production of seed stock for lobster culture. Aquaculture 44: 103–114.

AIKEN, D. E., S. L. WADDY, K. L. MORELAND, AND S. M. POLAR. 1984. Electrically induced ejaculation and artificial insemination of the American lobster *Homarus americanus*. J. Crustacean Biol. 4: 519–527.

AIKEN, D. E., S. L. WADDY, AND L. S. UHAZY. 1985. Aspects of the biology of *Pseudocarcinonemertes homari* and its association with the American lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 42: 351–356.

BUMPUS, H. C. 1891. The embryology of the American lobster. J. Morphol. 5: 215–263.

CONKLIN, D. E. 1983. Lobster culture: a perspective on intensive crustacean rearing, p. 875–879. *In* Proceedings, Oceans '83, Volume II: Technical Papers 83 CH1972-9, Marine Technology Society, San Francisco, CA.

DUNHAM, P. J., AND D. SKINNER-JACOBS. 1978. Intermolt mating in the American lobster (*Homarus americanus*). Mar. Behav. Physiol. 5: 209–214.

HARPER, R. E., AND P. TALBOT. 1984. Analysis of the epibiotic bacteria of lobsters (*Homarus*) eggs and their influence on the loss of eggs from the pleopods. Aquaculture 36: 9–26.

HEDGECOCK, D. 1983. Maturation and spawning of the American lobster, *Homarus americanus*, p. 261–270. *In* J. P. McVey [ed.] CRC handbook of mariculture. Vol. 1. Crustacean aquaculture. CRC Press, Boca Raton, FL.

TALBOT, P. 1984. Problems and progress in controlling reproductive biology in the American lobster (*Homarus*), p. 473–480. *In* W. Engels et al. [ed.] Advances in invertebrate reproduction 3. Elsevier Science Publishers, Amsterdam, The Netherlands.

TALBOT, P., AND R. HARPER. 1984. Abnormal egg stalk morphology is correlated with clutch attrition in laboratory-maintained lobsters (*Homarus*). Biol. Bull. 166: 349–356.

TALBOT, P., C. THALER, AND P. WILSON. 1984. Spawning, egg attachment and egg retention in captive lobsters (*Homarus americanus*). Aquaculture 37: 239–249.

WADDY, S. L., AND D. E. AIKEN. 1984. Seed stock for lobster culture: the role of temperature in synchronizing the molt and reproductive cycle of cultured American lobsters. J. World Maricult. Soc. 15: 132–137.

Potential of Parasites for Use as Biological Indicators of Migration, Feeding, and Spawning Behavior of Northwestern Atlantic Herring (*Clupea harengus*)

S. E. McGladdery[1] and M. D. B. Burt

Department of Biology, University of New Brunswick, Fredericton, NB E3B 6E1

McGladdery, S. E., and M. D. B. Burt. 1985. Potential of parasites for use as biological indicators of migration, feeding, and spawning behavior of northwestern Atlantic herring (*Clupea harengus*). Can. J. Fish. Aquat. Sci. 42: 1957–1968.

A parasite survey of Atlantic herring, *Clupea harengus*, collected from the Bay of Fundy, Nova Scotian Shelf, and Gulf of St. Lawrence, was conducted from May 1981 to November 1983. A total of 2380 herring were examined and 18 parasite species identified. These included the acanthocephalan *Echinorhynchus gadi* previously unreported from herring in the northwestern Atlantic and the monogenean *Gyrodactyloides andriaschewski* herein described from herring for the first time. The geographic distributions of the parasites, their seasonal dynamics, and relation to size of herring were recorded to determine whether they could be used as biological indicators, both to differentiate between individual herring populations and to determine their movements. Seven of the 18 species identified showed varying degrees of potential as biological indicators. Although no single parasite species was found which could clearly differentiate one population of herring from another, the differential prevalences and intensities of *Anisakis simplex*, *Hysterothylacium aduncum*, *Derogenes varicus*, *Lecithaster gibbosus*, *Cryptocotyle lingua*, *Scolex pleuronectis*, and *Eimeria sardinae* all provided valuable contributions to the knowledge already accumulated on herring migration, feeding, and spawning behavior. Parasite indicators, used in conjunction with other data and techniques, could eventually be used to distinguish between individual populations of herring.

Entre mai 1981 et novembre 1983, on a effectué une étude sur les parasites présents chez des harengs de l'Atlantique (*Clupea harengus*) capturés dans la baie de Fundy, sur la plate-forme de la Nouvelle-Écosse et dans le golfe du Saint-Laurent. On a examiné au total 2 380 harengs et l'on a identifié 18 espèces de parasites. Parmi ces derniers, on a trouvé l'acanthocéphale *Echinorhynchus gadi*, qui n'avait pas été signalé auparavant chez le hareng dans l'Atlantique Nord-Ouest, ainsi que le monogène *Gyrodactyloides andriaschewski* décrit dans le présent article, qui est signalé pour la première fois chez le hareng. On a recueilli des données sur la distribution géographique des parasites, sur leurs activités saisonnières et sur leur rapport avec la taille des harengs pour déterminer si on pouvait s'en servir comme indicateurs biologiques pour différencier les diverses populations de hareng et déterminer leurs déplacements. Sept des 18 espèces identifiées ont montré à des degrés divers qu'on pouvait les utiliser comme indicateurs biologiques. Bien qu'on n'ait pas trouvé une espèce particulière de parasite qui permette de distinguer nettement une population de hareng d'une autre, les différences observées dans la fréquence et la quantité des espèces *Anisakis simplex*, *Hysterothylacium aduncum*, *Derogenes varicus*, *Lecithaster gibbosus*, *Cryptocotyle lingua*, *Scolex pleuronectis* et *Eimeria sardinae* ont permis d'accroître les connaissances qu'on possède déjà sur la migration, l'alimentation et le comportement reproducteur des harengs. Les indicateurs biologiques que constituent les parasites, utilisés de concert avec d'autres données et techniques, pourraient éventuellement servir à différencier entre elles les populations de hareng.

Received February 21, 1985
Accepted August 8, 1985
(J8124)

Reçu le 21 février 1985
Accepté le 8 août 1985

One of the main problems currently facing fishery biologists from the northwestern Atlantic is the identification of and differentiation between individual populations of Atlantic herring (*Clupea harengus*). A number of techniques have been applied to solve this problem, e.g. tagging (Anthony 1981a; Stobo 1983; Waring 1981), morphometrics and meristics (Anthony 1981b; Lough 1976; Parsons 1972, 1973; Parsons and Hodder 1974), and electrophoretic studies (Odense and Annand 1980; Truveller 1971; Kornfield et al. 1982; Grant 1984). Although these studies have all provided valuable information, the problem of differentiating between individual herring populations persists, and in order to increase management efficiency, additional methods that can supplement the data provided by present techniques are required.

One method, involving the use of parasites as biological indicators to differentiate between individual populations of fish, has recently gained increased recognition as being applicable to the problems of fishery management. Compared with artificial tags, biological indicators (i) are less expensive, (ii) do not involve catch-and-release methods which are known to increase the rate

[1]Present address: Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB E1C 9B6.

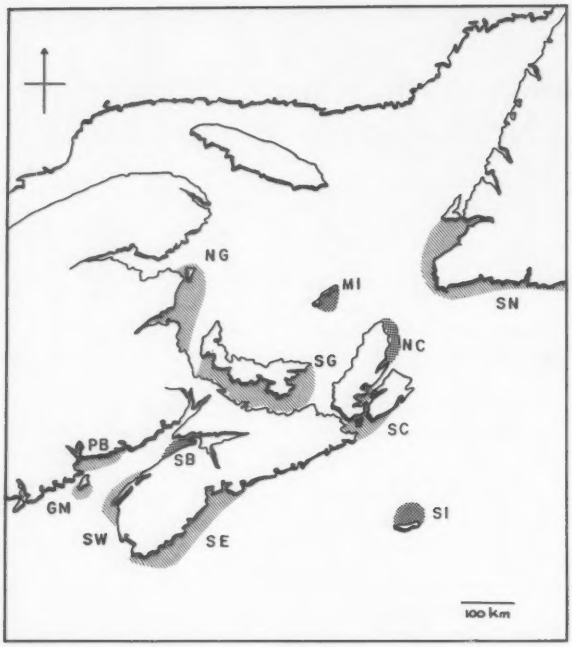


FIG. 1. Areas sampled for northwestern Atlantic herring (*Clupea harengus*). Main sampling areas (shaded): PB, Passamaquoddy Bay; GM, Grand Manan; SW, southwest Nova Scotia; SE, southeast Nova Scotia; SC, southern Cape Breton; SN, southern Newfoundland; SG, southern Gulf of St. Lawrence; NG, northern Gulf of St. Lawrence. Additional sampling areas (dotted): SB, Scots Bay; SI, Sable Island Bank; NC, northern Cape Breton; MI, Magdalen Islands.

of mortality of tagged fish (Parker et al. 1963; Jones 1977), and (iii) monitor the movements of populations of fish, rather than tagged individuals. In addition, certain parasites can provide information on the overall biology of their fish host (e.g. feeding behavior, spawning behavior, and recruitment patterns) as well as on their migration patterns.

Biological indicators have been used successfully on other fish species (Gibson 1972; Hare and Burt 1976; Hislop and MacKenzie 1976; Khan et al. 1982; Lubieniecki 1977; Margolis 1963; MacKenzie and Mehl 1984; Olson and Pratt 1973; Platt 1976; Scott 1969, 1975, 1981; Sherman and Wise 1961; Shotter 1973) as well as on herring from the northeastern Atlantic (MacKenzie 1974, 1975; MacKenzie and Johnson 1976). Sindermann's (1957a, 1957b) work on the geographic distribution of parasites of herring along the east coast of the United States and from southern Nova Scotia showed that two species (a myxosporean, *Kudoa clupeidae*, and larvae of the nematode *Anisakis simplex*) were of potential use as biological indicators. The objective of our study was to determine if parasite species that could be useful as biological indicators exist in herring from the east coast of Canada.

Materials and Methods

Between May 1981 and November 1983, 2380 herring, collected from various locations around the east coast of Canada (Fig. 1), were examined for their parasites. Initially, each sample consisted of 20 fresh herring which were supplemented, wherever possible, by an additional 20 frozen herring. After the first year of investigation, because sufficient fresh parasite specimens had been collected to permit accurate identification, the basic sample size was increased to 40 herring, all of which were frozen. Three size-groups of herring were examined: (i) "sardines" aged 0+ to 1+ yr and approximately 4–12 cm long (fork length); (ii) juveniles aged 2 to 3+ yr and approximately 12–24 cm long; and (iii) adults aged from 3 yr upwards approximately 24–40 cm long. Wherever possible, samples of each size-group were collected monthly.

Live platyhelminth specimens were fixed in hot 10% formalin, left for approximately 24 h, and stored in 70% ethanol. Live nematodes and acanthocephalans were fixed in hot 70% ethanol and stored in 9 parts 70% ethanol and 1 part glycerine. Dead platyhelminths were fixed and stored in 70% ethanol and dead nematodes and acanthocephalans were fixed and stored in cold 70% ethanol + 10% glycerine.

Platyhelminths were stained in either acetic acid alum carmine or Semichon's carmine, cleared in xylene, and mounted in Permount for detailed examination. Nematodes were cleared in glycerine or lactophenol and mounted temporarily in glycerine jelly for detailed examination.

Statistical Analysis

Nonparametric statistics were used for all data analyses, since Bartlett's test for homogeneity of variances (Sokal and Rohlf 1969) showed significant differences ($p < 0.05$) among samples. Chi-squared analyses were used, where applicable, to test for significant differences among prevalences of individual parasite species. The general linear model (GLM) procedure was used for analysis of variance (ANOVA), among samples of different size-groups of herring and among samples collected from different locations and at different times of year. Raw intensity data (mean number of parasites per infected fish) were transformed by square root ($y = \pm\sqrt{x + 0.5}$) to avoid bias due to exceptional infections. A probability less than 0.05 represented a significant difference in all ANOVAS using Duncan's multiple range test. The term "prevalence," as used in the text, is defined as the percentage of individuals in a sample infected by a particular parasite species. The term "abundance" is defined as the mean number of a particular parasite species per host examined (Margolis et al. 1982).

Results

Eighteen parasite species were identified from the herring examined (Table 1). Only seven species were sufficiently common, with consistent differences in prevalence between geographic areas, seasons, and size-groups to show potential as biological indicators for herring from the east coast of Canada.

Anisakis simplex (Rudolphi, 1809) Dujardin, 1845

Third stage larvae of the "herring worm" *Anisakis simplex* were found in herring from all sites sampled around the east coast of Canada.

Prevalence of *A. simplex* larvae increased with herring age (Fig. 2), with a significant ($p < 0.05$) difference in prevalence between 2-yr-old and older herring. Intensity of infection increased in herring with increased age between 3 and 9 yr old. However, the infection decreased significantly ($p < 0.05$) in herring over 10 yr old. No significant differences ($p < 0.05$) in prevalence or intensity of infection were found in relation to

TABLE 1. Parasites recovered from sardine (S), juvenile (J), and adult (A) *Clupea harengus*.

Parasite species	Principal location within herring	Size-groups infected	Parasite stage	Distribution
Nematoda: Anisakidae				
Anisakis simplex	Body cavity, mesenteries, musculature	J, A	Larva	Variable
Hysterothylacium aduncum	Pyloric mesenteries	S, J, A	Larva	Variable
Acanthocephala: Echinorhynchidae				
Echinorhynchus gadi[a]	Intestine	J, A	Preadult	Rare
Monogenea: Gyrodactylidae				
Gyrodactylus harengi	Gills	S, J, A	Adult	Uniform
Gyrodacyloides andriacshewi[b]	Gills	S, J, A	Adult	Uniform
Digenea: Hemiuridae				
Brachyphallus crenatus	Stomach	S, J, A	Adult	Uniform
Derogenes varicus	Stomach	S, J, A	Adult	Variable
Lecithaster gibbosus	Intestine	S, J, A	Adult	Variable
L. confusus	Intestine	S, J, A	Adult	Rare
Hemiurus levinseni	Stomach	A	Adult	Rare
Digenea: Heterophyidae				
Cryptocotyle lingua	Skins, fins	S, J, A	Metacercariae	Variable
Cestoda: Tetraphyllidea				
Scolex pleuronectis	(I) intestine;	S, J, A	Metacestode	Variable
	(II) pyloric caeca, intestine, stomach	S, J, A	Metacestode	Rare
Cestoda: Trypanorhyncha				
Grillotia erinaceus	Intestinal mesenteries	A	Metacestode	Rare
Lacistorhynchus tenuis	Pyloric mesenteries	A	Metacestode	Rare
Apicomplexa: Coccidia				
Eimeria clupearum	Liver	S, J, A	Oocyst	Uniform
E. sardinae	Testes	J, A	Oocyst	Variable
Myxozoa: Ceratomyxidae				
Ceratomyxa auerbachi	Gallbladder	S, J, A	Sporocyst	Rare

[a]New location record.
[b]New host record.

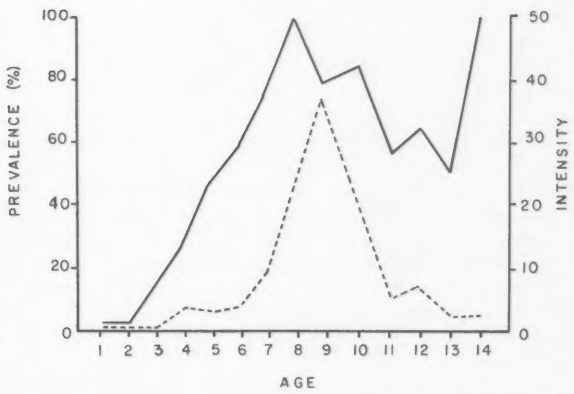


FIG. 2. Prevalence (—) and mean intensity (---) of infection by *Anisakis simplex* in herring of different ages.

season; however, *A. simplex* larvae showed consistent variation in geographic distribution. Since the prevalence in juvenile and sardine-size herring is lower than in adults (Fig. 2), and no smaller herring were collected from Newfoundland or the Gulf of St. Lawrence, the geographic distribution of *A. simplex* is best illustrated by the prevalences of infection found in adult herring (Fig. 3). Herring in the Bay of Fundy consistently had lower prevalences of *A. simplex* than herring caught at the other locations sampled. The highest prevalence (100%) was found in herring collected from southern Newfoundland in winter. The only other sample that was as heavily infected was from Sable Island Bank in summer. The prevalences found in the Gulf of St. Lawrence ($\bar{x} = 57.3 \pm 14.0\%$) were also high but not as high as those off southern Newfoundland ($\bar{x} = 84.3 \pm 15.0\%$). The prevalences of *A. simplex* found in herring off southern Nova Scotia did not exceed 50% but were consistently greater than those found within the Bay of Fundy.

Hysterothylacium aduncum (Rudolphi, 1802) Deardorff and Overstreet, 1981

Third and fourth stage larvae of the anisakine nematode *Hysterothylacium aduncum* were found in herring at all locations sampled. Adult *H. aduncum*, however, were rarely found (five specimens only). In direct contrast with *A. simplex*, *H. aduncum* decreased in both prevalence and, to a lesser extent, intensity of infection as herring increased in age (Fig. 4). There was no clear correlation, however, between prevalence or intensity of infection and season. The geographic distribution of *H. aduncum* is shown in Fig. 5. The prevalences found at the

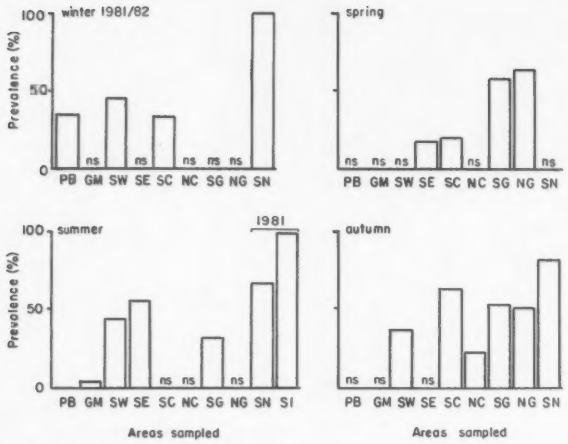


FIG. 3. Prevalence of *Anisakis simplex* in adult herring for each season in 1982 (except where indicated 1981). Key to areas sampled given in Fig. 1. Areas where no samples were collected are indicated by "ns".

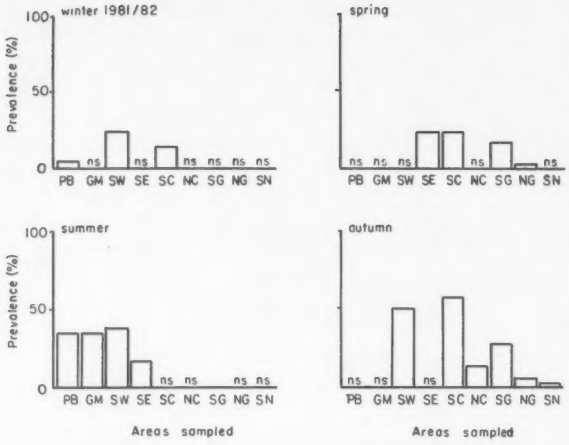


FIG. 5. Prevalence of *Hysterothylacium aduncum* in adult herring for each season in 1982. Key to areas sampled given in Fig. 1. Areas where no samples were collected are indicated by "ns".

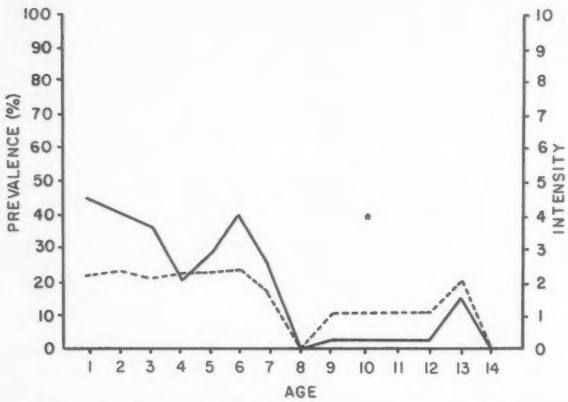


FIG. 4. Prevalence (—) and mean intensity (---) of infection by *Hysterothylacium aduncum* in herring of different ages (*denotes a single herring with four worms).

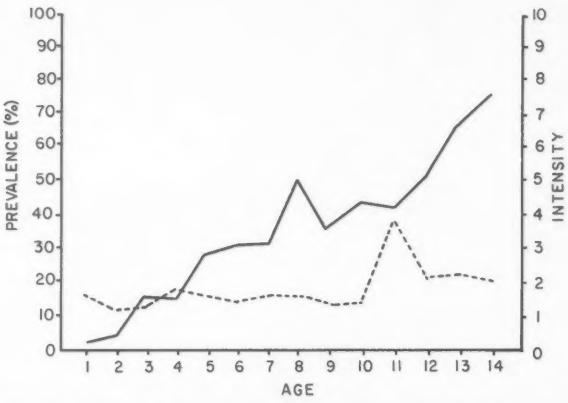


FIG. 6. Prevalence (—) and mean intensity (---) of infection by *Derogenes varicus* in herring of different ages.

various sampling localities showed consistent differences, the highest being found in herring collected from the Bay of Fundy and around southern Nova Scotia. The prevalences of *H. aduncum* further north tended to be lower, with the exception of those off Cape Breton (57.5%) and, to a lesser extent, in the southern Gulf of St. Lawrence (30.3%), in autumn. The prevalence off Cape Breton in autumn was not significantly different ($p > 0.05$) from those off southern Nova Scotia in summer (46%) and autumn (50%).

Derogenes varicus (Müller, 1784) Looss, 1901

The hemiurid digenean *Derogenes varicus* was found as an adult in the stomach of herring. This parasite showed an increase in prevalence with increased herring age (Fig. 6). There is no correlation between intensity of infection and age, however, so the increase in prevalence of *D. varicus* is unlikely to be due to accumulation of long-lived infections. Evidence for the limited life span of *D. varicus* is provided by the seasonal fluctuation in prevalence (Fig. 7), which peaks in spring and summer. The lack of increase in intensity of infection also indicates that adult *D. varicus* do not survive long enough to be accumulated through repeat infections, although some can survive over winter in herring (pers. obs.). The large standard deviations (SD) of the mean reflect the unusually high prevalences found in herring from southern Newfoundland.

Most herring over 11 yr old were collected from the Gulf of St. Lawrence or around southern Newfoundland, and the higher prevalences from these areas (Fig. 8) probably account for the high prevalences found in older herring. The highest prevalences of this parasite were consistently found off southern Newfoundland, with all other localities, except Cape Breton in spring (52.5%) and Sable Island Bank in summer (54%), having prevalences less than 40%.

Lecithaster gibbosus (Rudolphi, 1802) Lühe, 1901

This digenean, found as an adult in the intestine and, to a lesser extent, in the pyloric ceca, showed pronounced seasonal fluctuations both in prevalence and intensity of infection (Fig.

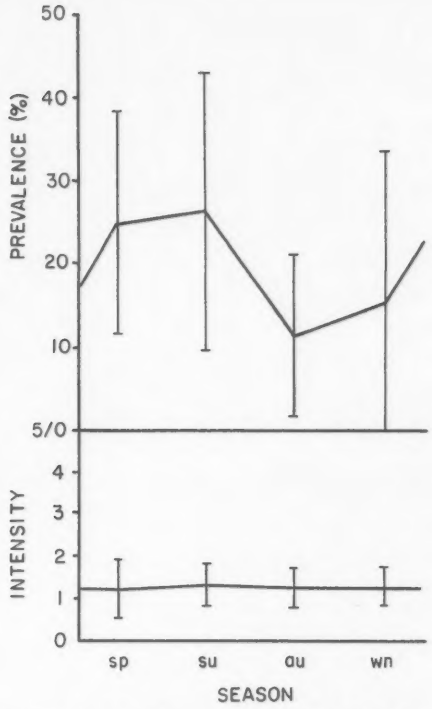


FIG. 7. Prevalence and mean intensity of infection by *Derogenes varicus* in all sizes of herring collected in spring (sp), summer (su), autumn (au), and winter (wn), 1982. Vertical lines = ±1 SD.

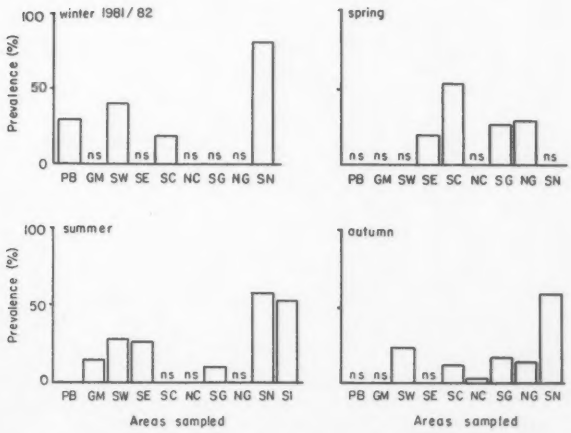


FIG. 8. Prevalence of *Derogenes varicus* in adult herring for each season in 1982. Key to areas sampled given in Fig. 1. Areas where no samples were collected are indicated by "ns".

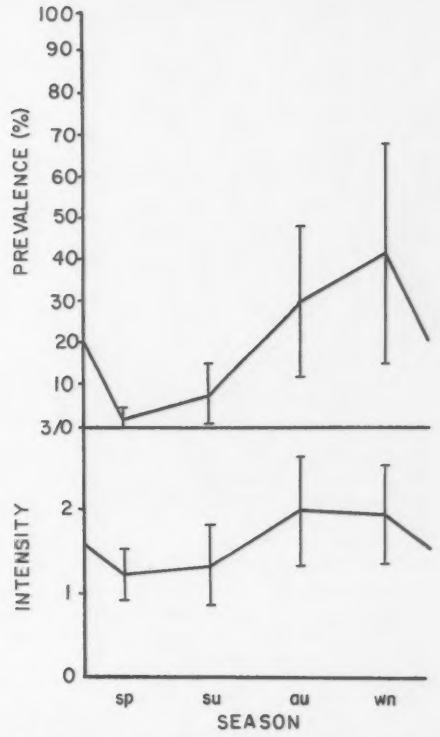


FIG. 9. Prevalence and mean intensity of infection by *Lecithaster gibbosus* in all sizes of herring collected in spring (sp), summer (su), autumn (au), and winter (wn), 1982. Vertical lines = ±1 SD.

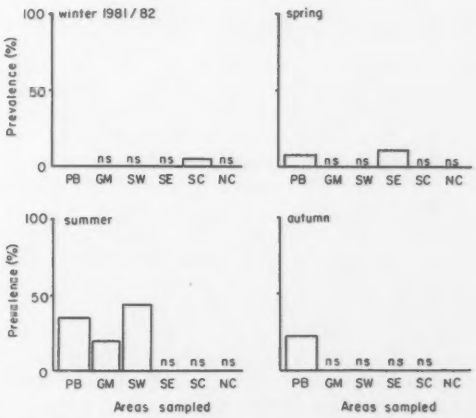


FIG. 10. Prevalence of *Lecithaster gibbosus* in juvenile herring for each season in 1982. Key to areas sampled given in Fig. 1. Areas where no samples were collected are indicated by "ns".

9). A peak of infection occurred in autumn followed by a decline over winter, with the lowest prevalences and intensities of infection having occurred in spring. When the infections found in juvenile and adult herring were examined separately, however, two different peaks of infection were found. Juvenile herring showed a peak of infection in summer (Fig. 10) whilst adults showed this peak of infection in autumn (Fig. 11). There was no overall correlation, however, between prevalence or intensity of infection and age of herring, which indicates that the life span of *L. gibbosus* in herring is <1 yr. The significant decrease ($p < 0.05$) in prevalence between winter and spring (Fig. 9) suggests further that most *L. gibbosus* do not overwinter in herring. Juvenile herring with natural infections of *L. gibbosus* were kept in holding tanks between July 1983 and January 1984 and fed freeze-dried pellet food. These herring showed a decrease in prevalence of *L. gibbosus* from 52.5% in July to 5%

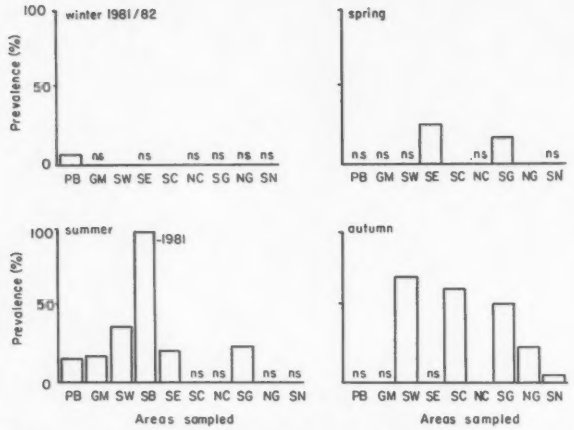


FIG. 11. Prevalence of *Lecithaster gibbosus* in adult herring for each season in 1982 (except where indicated by 1981). Key to areas sampled given in Fig. 1. Areas where no samples were collected are indicated by "ns".

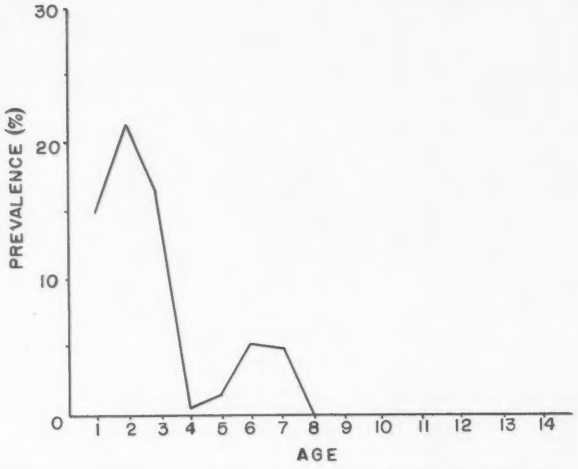


FIG. 12. Prevalence of *Cryptocotyle lingua* in herring of different ages.

in October. The mean intensity of infection decreased from 2.5 to 1.0 over the same period. Some specimens, possibly acquired by the herring late in the season, appear able to overwinter in herring, but most of the infections, possibly acquired in spring, were lost by September and October.

An unusually high prevalence (95%) and intensity of infection (12.1 ± 17.3) with *L. gibbosus* was found in a sample of adult herring collected from Scot's Bay in summer, 1981 (Fig. 11, SB). The only other sample that showed a similar prevalence (80%) and intensity of infection (10.0 ± 8.0) was of sardines collected from southwest Nova Scotia over the same period.

Cryptocotyle lingua (Creplin, 1825) Fischoeder, 1903

Metacercariae of this digenean were most commonly found encysted in the skin of younger herring (Fig. 12), and no herring over 8 yr old were infected. There was a significant decrease ($p < 0.05$) in prevalence in herring between 3 and 4 yr old, which corresponds with the age when these fish start to feed offshore on deeper-water zooplankton (Maurer 1976) (*C. lingua* has an inshore life cycle). The sharp decrease in prevalence in fish over 4 yr old also suggests that *C. lingua* metacercariae have a short life span, once encysted in herring, and further evidence of this is provided by the marked seasonal fluctuations in prevalence (Fig. 13).

The geographic distribution of *C. lingua* in herring 4–8 yr old is given in Fig. 14. The highest prevalences occurred in the Bay of Fundy in summer and autumn. All other samples of adult herring had prevalences less than 25%.

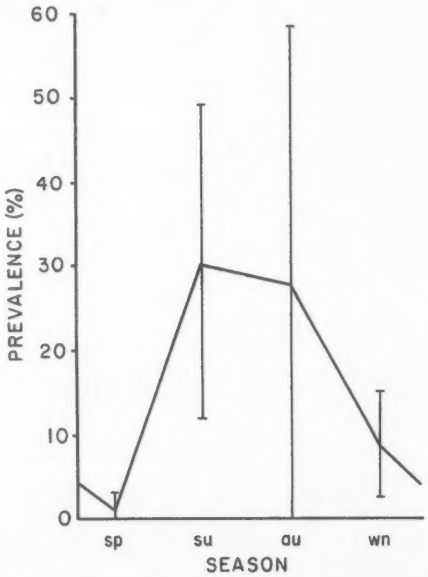


FIG. 13. Prevalence of *Cryptocotyle lingua* in all sizes of herring collected in spring (sp), summer (su), autumn (au), and winter (wn), 1982. Vertical lines = ±1 SD.

Scolex pleuronectis O. F. Müller, 1788

Two forms of these tetraphyllidean larvae were found: (i) a "microscopic" form, characterized by four unilocular suckers and measuring 0.12–0.27 mm in length by 0.079–0.085 mm wide, and (ii) a "macroscopic" form, characterized by four bilocular suckers and measuring 1.02–2.24 mm in length by 0.35–0.50 mm wide. Some specimens of the larger form have a pink ring or four pink patches at the base of each bothridium.

The microscopic form of *S. pleuronectis* occurred too infrequently to provide detailed information on its geographic or seasonal distribution. The macroscopic form, however, had an interesting geographic distribution occurring, with only one exception, in the Bay of Fundy and around southern Nova Scotia. The exception was a single specimen taken from an adult herring collected in the northern Gulf of St. Lawrence in summer, 1981. The distribution of the macroscopic form of *S. pleuronectis* is shown in Fig. 15. There was little correlation between prevalence or intensity of infection and age of herring. There was, however, pronounced seasonal fluctuation in prevalence, with the peak occurring in summer (Fig. 16).

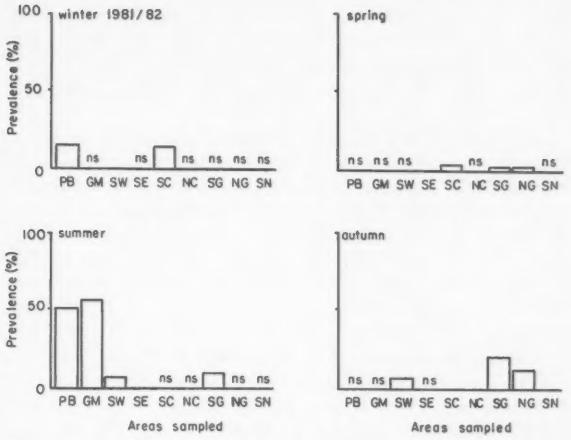


FIG. 14. Prevalence of *Cryptocotyle lingua* in adult herring for each season in 1982. Key to areas sampled given in Fig. 1. Areas where no samples were collected are indicated by "ns".

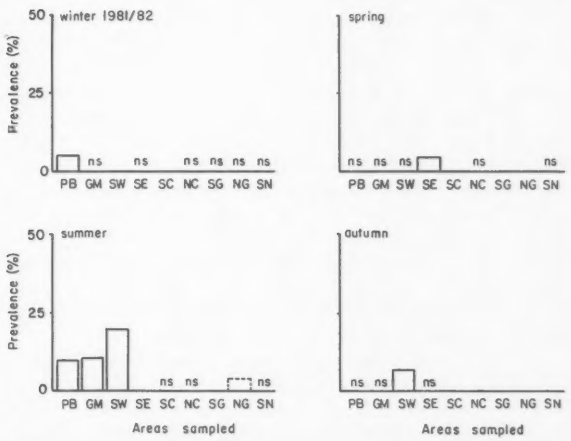


FIG. 15. Prevalence of *Scolex pleuronectis* (macroscopic form) in adult herring for each season in 1982. Key to areas sampled given in Fig. 1. Areas where no samples were collected are indicated by "ns".

Eimeria sardinae (Thélohan, 1890) Reichenow, 1921

The oocyst stages of this protozoan were found in the testes of male herring. No other stages in the life cycle of this parasite could be detected with the squash technique used in this investigation. Despite this limitation, however, the prevalence of the oocyst stages showed a strong correlation with the stage of maturity of the gonads (for maturity stages see Parrish and Saville 1965) (Fig. 17). There was a significant increase ($p < 0.05$) in prevalence between the resting stage (III) and ripe stage (V) of maturation. The highest prevalence was found in spawning herring (stage VI) and this was followed by a significant decrease ($p < 0.05$) in spent herring (stages VII and VIII). This decrease corresponds to the release of the oocysts with sperm during spawning. Immature herring (stages I and II) were rarely infected with *E. sardinae* oocysts.

The geographic distribution of *E. sardinae* oocyst infections in adult herring is shown in Fig. 18. At all locations sampled, the

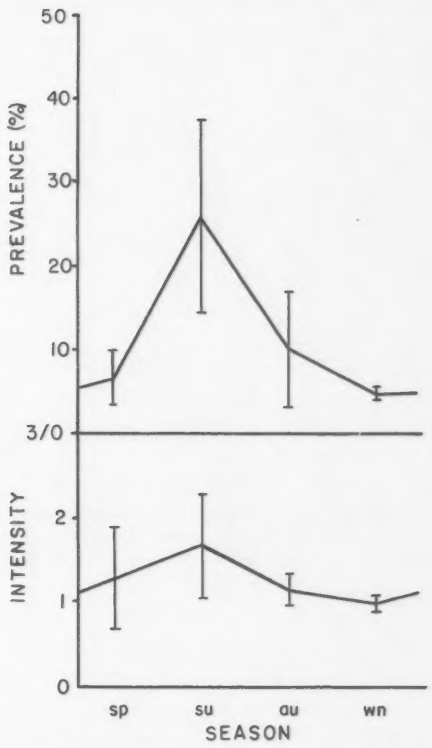


FIG. 16. Prevalence and mean intensity of infection by *Scolex pleuronectis* (macroscopic form) in all sizes of herring collected in spring (sp), summer (su), autumn (au), and winter (wn), 1982. Vertical lines = ±1 SD.

prevalences found showed seasonal variations which correlated with the maturation of spring- and autumn-spawning herring.

Different Aspects of the Biology of Herring Indicated by Their Parasites

Each of the seven parasite species investigated provided information that could be used to differentiate between one or more aspects of their herring host's biology, i.e. patterns of migration, feeding, and spawning behavior.

Patterns of herring movement suggested by parasite indicators

Movement into and out of the Bay of Fundy — Movements into and out of the Bay of Fundy were indicated by *A. simplex*, *H. aduncum*, *C. lingua*, *S. pleuronectis*, and *E. sardinae*. The lowest prevalences of the nematode *A. simplex* were consistently found in herring collected from the Bay of Fundy (Fig. 3), although there was a slight increase in prevalence at this locality over autumn and winter. This increase, however, was unlikely to be due to new infections, since adult herring in this area spawn in autumn and do not feed over this period (Pankratov and Sigajev 1974). The increase in prevalence over autumn and winter must, therefore, be due to an influx of more heavily infected herring into the Bay of Fundy, such as those from southern Nova Scotia, which consistently had higher prevalences. It is unlikely that the more commonly infected herring came from further south, since herring collected in the Gulf of Maine had similar prevalences to those collected from the New Bruns-

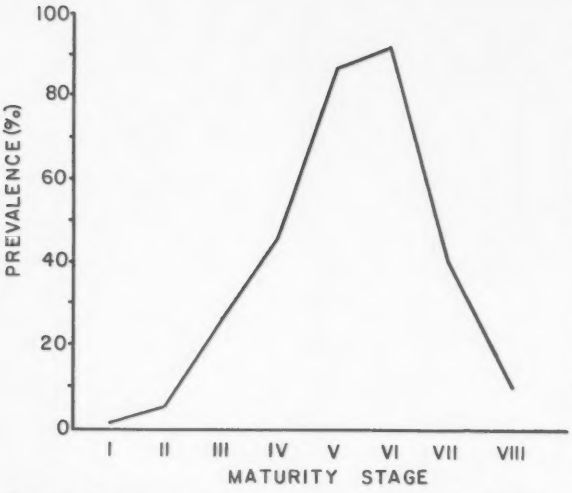


FIG. 17. Prevalence of *Eimeria sardinae* oocysts in male herring with gonads at different maturity stages. Stages I–II, immature; III–IV, prespawning; V–VI, ripe and running; VII–VIII, spent.

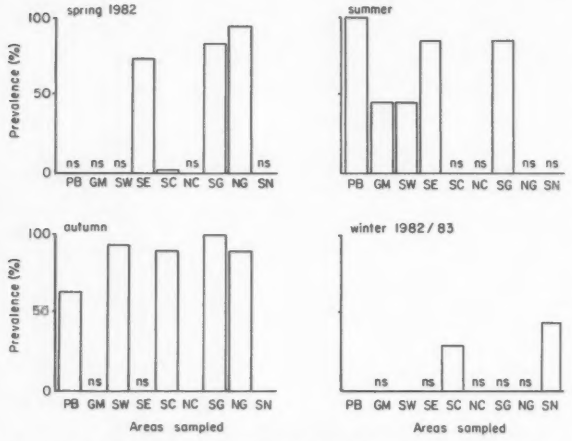


FIG. 18. Prevalence of oocysts of *Eimeria sardinae* in adult male herring for each season in 1982. Key to areas sampled given in Fig. 1. Areas where no samples were collected are indicated by "ns".

wick shore of the Bay of Fundy (Boyar and Perkins 1971; Chenoweth and S. E. McGladdery, unpubl. data).

The possibility of an inshore migration of adult herring from the Scotian shelf is reinforced by the decrease in prevalence shown by the nematode *H. aduncum* over the same period (Fig. 5). This nematode is found commonly in "sardine" and juvenile herring that spend more time in shallower water than do adults and that feed predominantly on copepod species whilst adults feed on larger crustaceans, such as euphausiids. Copepods and smaller crustaceans are known to be the most common first intermediate hosts of *H. aduncum* (Scott 1957; Smith 1983), and therefore, the decrease in prevalence over winter may be due, in part, to an influx of less heavily infected herring from offshore. Part of the decrease may also have been due to loss of larvae which die whilst within the body cavity of the fish. The lack of a clear seasonal variation in prevalence and intensity of infection and the observation of dead and degenerate larvae in herring all year round, however, suggest that the winter decrease in prevalence of infection in the Bay of Fundy is not due entirely to the limited life span of these larvae in infected fish.

A third, completely unrelated parasite that also indicates a movement of offshore herring into the Bay of Fundy to overwinter is the digenean *C. lingua*. As with the nematode *H. aduncum*, *C. lingua* has an inshore life cycle, being transmitted to herring by free-swimming larval stages released from the inshore periwinkle, *Littorina littorea* (Stunkard 1930). The prevalence of *C. lingua* in the Bay of Fundy in summer was over 50% (Fig. 14). In winter, however, the prevalence decreased to 17%. Since *C. lingua* metacercariae can survive for over a year in their herring hosts, it is unlikely that this decrease in prevalence over winter is due to death and loss of these larvae, especially when the persistence of the melanised cyst, after the larva has died (pers. obs.), is taken into account. The decrease must, therefore, be due to an influx of uninfected herring into the Bay of Fundy.

The winter influx of adult herring into the Bay of Fundy is also suggested by a fourth potential biological indicator, the macroscopic form of the tetraphyllidean plerocercoid *S. pleuronectis*. These metacestodes show a more or less discrete distribution, being found most commonly in herring from the Bay of Fundy and around southern Nova Scotia (Fig. 15). The only herring carrying *S. pleuronectis* metacestodes during winter were in Passamaquoddy Bay. In spring, adults move away from this area, and since the only area that showed an increase in infection was southeast Nova Scotia, and the prevalence found there in spring was the same as that in Passamaquoddy Bay in winter, this indicates that at least some adult herring leave Passamaquoddy Bay in spring and move to southeast Nova Scotia. It is unlikely that the increase in infection off southeast Nova Scotia in spring is due to new infections, since the adjacent areas of Grand Manan and southwest Nova Scotia showed no such increase over the same period.

Between autumn and winter, there is a significant decrease ($p < 0.05$) in prevalence of *S. pleuronectis* which may be due to loss of older infections. However, some metacestodes have been shown to survive over winter (McGladdery 1984), and this decrease may, therefore, also be due to an influx of uninfected herring into the area.

Another parasite that indicates a movement away from the Bay of Fundy in spring is the protozoan *E. sardinae*, found in the testes. In March 1982 and 1983, the prevalence of this parasite in herring collected from southern Nova Scotia was high (Fig. 18), indicating that they were spring spawners. Since there are no known spring spawning grounds in that area, it seems likely that these herring are en route north, towards spawning grounds in the Bras d'Or lakes of Cape Breton or in the southern Gulf of St. Lawrence, possibly after overwintering in the Bay of Fundy. No evidence is provided for movement in the opposite direction into the Bay of Fundy and towards summer spawning grounds at Scots Bay. Both *A. simplex* and *S. pleuronectis* indicate movement of adults out of the Bay of Fundy in spring, rather than vice versa.

Movement to and from southern Newfoundland — Another migration route suggested by parasite indicators is a spring and summer movement of adult herring from southwest Newfoundland offshore, possibly to Sable Island Bank.

The highest prevalences of the nematode *A. simplex* were found in adult herring off the south shore of Newfoundland in

winter and on Sable Island Bank in summer (Fig. 3). After these herring overwinter off southern Newfoundland, the fishery in that area closes and it is assumed the herring move away. Since the prevalences and intensities of *A. simplex* in herring from Cape Breton and the Gulf of St. Lawrence, in spring, were not large enough to account for an influx of the heavily infected herring found off southern Newfoundland in winter, it seems likely that these heavily infected nsh belong to a discrete stock that moves offshore, possibly to a summer feeding ground on Sable Island Bank.

The suggestion of a spring movement offshore from southern Newfoundland is also indicated by the digenean *D. varicus*. This parasite showed exceptionally high prevalences and intensities of infection in herring from southern Newfoundland (Fig. 8), especially in winter. The only other areas that had prevalences of infection greater than 50% were Cape Breton and Sable Island Bank. In addition to the high prevalence of *D. varicus* in these areas, the intensities of infection were also higher than those found elsewhere (range 2.2–2.7 and 1.0–1.8, respectively), indicating a possible relationship between the herring stocks of southern Newfoundland, Cape Breton, and Sable Island Bank. If the increase in prevalence of *D. varicus* off Cape Breton, from 22.5% in winter to 52.5% in spring, was due entirely to new infections, similar increases would be expected at the other localities sampled. Since this clearly is not the case, the large increase in prevalence of *D. varicus* off Cape Breton in spring must be due to an influx of heavily infected herring, such as those from southern Newfoundland.

The absence of a similar increase in *A. simplex* off Cape Breton in spring is expected (Fig. 3). Juvenile herring overwinter off Cape Breton mixed in with adult fish. Since younger herring characteristically have low prevalences and intensities of *A. simplex* infections, these would be expected to dilute the high prevalence of infection found in the older herring moving from southern Newfoundland to Cape Breton. Only third stage larvae of *A. simplex* occur in herring and they are usually acquired by ingestion of infected euphausiids commonly found in deeper, offshore water. The digenean *D. varicus*, however, has an inshore life cycle (Køie 1979), with the adult stage occurring in all age-groups of herring (Fig. 6). An influx of herring heavily infected by *D. varicus*, combined with the infections already present in the overwintering Cape Breton fish, therefore, would be expected to cause an overall increase.

In summer, the adult herring off Cape Breton move away, and since the increase in prevalence of *D. varicus* off southern Nova Scotia is not large enough to account for the heavily infected herring found off Cape Breton in spring (Fig. 8), this suggests that the latter move offshore. There is an increase in prevalence of *D. varicus* off southern Newfoundland in summer. However, this is not as great as the prevalence of infection found there in winter and is more likely to be due to newly acquired infections than a return of the herring which overwintered off southern Newfoundland and moved away in spring.

Movement into and out of the Gulf of St. Lawrence — The increase in prevalence of *D. varicus* off southern Newfoundland in summer may indicate that in addition to the appearance of new infections, some herring from the Gulf of St. Lawrence move to this area. Herring from the Gulf of St. Lawrence showed a decrease in prevalence in summer when feeding behavior would be expected to produce an increase of *D. varicus* (Fig. 7). Therefore, there must be (i) an emigration of infected herring from the Gulf of St. Lawrence or (ii) an influx of uninfected fish. The suggestion of movement out of the Gulf of St. Lawrence by infected herring is also indicated by *A. simplex* which shows an increase in prevalence off southern Newfoundland in summer (Fig. 3) that is very similar to that found in the Gulf of St. Lawrence in spring. The summer movement of some herring out of the Gulf of St. Lawrence is also indicated by the protozoan *E. sardinae*. The high prevalence found in the Gulf of St. Lawrence in summer (Fig. 18) indicates that some spring-spawning herring had left. If a large number of spring spawners had been present in the summer feeding aggregations, the overall prevalence of *E. sardinae* would be expected to be lower. At the same time, the absence of *E. sardinae* in adult herring caught off southern Newfoundland indicates that they were all spring-spawning fish, some of which may have spawned in the Gulf of St. Lawrence.

Changes in patterns of herring movement — The nematode *A. simplex* also demonstrated an interesting correlation with the age of its host. The longevity of this parasite could be used to provide information on changes in patterns of herring movement in previous years.

Intensity of infection with *A. simplex* increased with age of hosts up to the age of 9 yr due, undoubtedly, to accumulation of successive infections. Herring over 10 yr old, however, showed a significant decrease in intensity of infection (Fig. 2) whilst prevalence decreased only slightly. The decrease in intensity of infection may simply have been a reflection of the smaller sample size of these older herring, or it may have been due to other factors: (i) the lower intensity of infection in herring over 10 yr old may reflect a loss of larvae that have died and degenerated; however, evidence for this is rare (i.e. degenerate *A. simplex* larvae); (ii) there may be a higher rate of mortality in more heavily infected older herring; or (iii) there may have been a lower rate of infection early in the adult life of older herring, compared with subsequent years. These fish would, therefore, be expected to exhibit similar or lower intensities of infection than those of younger adult herring, which were subject to a relatively constant rate of infection. This change in rate of infection could indicate either (i) a change in migration pattern of the older herring (Banning and Becker 1978) which increases their exposure to *A. simplex* larvae or (ii) a change in the population size of other hosts in the life cycle of *A. simplex*, such as the euphausiid intermediate hosts or cetacean definitive hosts.

The longevity of *A. simplex* larvae in herring, therefore, means that they have the potential to indicate past migration patterns of their fish hosts, especially in areas where the population dynamics of the other hosts involved in their life cycle are known.

Differences in diet of adult and younger herring indicated by parasites

Herring are pelagic fish, feeding mainly on planktonic crustaceans. They are active particulate feeders even at the larval stage, and although primarily opportunistic, they do show a clear progression from eating smaller to larger species of zooplankton as they increase in size (Sherman and Perkins 1971; Maurer 1976). The changes in diet recorded from stomach content analyses are also indicated by differences in parasite burden in the different size-groups of herring.

The nematode *A. simplex* showed a significant increase ($p < 0.05$) in prevalence of infection in herring between ages 2 and 3 (Fig. 2). This corresponds to the age when herring change from a predominantly copepod diet to a predominantly euphausiid diet (Sherman and Perkins 1971; Maurer 1976). Euphausiids are

the most common first intermediate hosts of *A. simplex* (Smith 1983). Once acquired, these larvae can survive for as long as their fish host (Banning and Becker 1978). Therefore, samples of herring that have been feeding on euphausiids in areas where *A. simplex* infections are common (such as the Gulf of St. Lawrence), but are uninfected by *A. simplex* or have light infections only, can be assumed to have only recently started feeding on these crustaceans. Conversely, herring heavily infected by *A. simplex*, but with copepod stomach contents, can be assumed to have fed on euphausiids previously.

In direct contrast with *A. simplex*, the heaviest infections of *H. aduncum* occurred in younger herring (Fig. 4), although the correlation with age of host is less marked. The less pronounced correlation with age of host is due to the fact that older herring can feed on large numbers of copepods in addition to their predominantly euphausiid diet whilst younger herring can ingest a limited number of euphausiids only. The probability of an adult ingesting a copepod infected with *H. aduncum* is, therefore, greater than that of a juvenile ingesting a euphausiid infected with *A. simplex*. Although adults can still acquire *H. aduncum*, they do not accumulate the prevalences or intensities of infection found in younger herring which feed more extensively on the intermediate hosts.

A third parasite that indicates the difference between the diets of juvenile and adult herring is the digenean *L. gibbosus*. Unlike the two nematodes, this parasite showed little difference in prevalence or intensity of infection in different sizes of herring. However, when the seasonal prevalences of infection were examined separately for adult and juvenile herring, there was a clear difference in the time of peak infection. Juvenile herring showed the peak in prevalence of *L. gibbosus* in summer (Fig. 10) whilst this did not occur in adult herring until autumn (Fig. 11). The later peak in prevalence in adults was due, in part, to recruitment of heavily infected, virgin spawners into the adult stock in autumn, prior to spawning. The later peak in prevalence of *L. gibbosus* in adults is also indicative of their ingesting fewer second intermediate hosts of *L. gibbosus*, e.g. copepods (Margolis and Boyce 1969), than juvenile herring. Infections of this parasite would, therefore, be expected to be accumulated more slowly by adult herring.

Spawning behavior indicated by parasites

The only parasite that was of use for indicating spawning behavior was the protozoan of the testes, *E. sardinae*. The close correlation between the development of the oocyst stage of this parasite and the maturation of the gonads (Fig. 17) provides information that can be used to distinguish spring- and autumn-spawning herring during periods when their gonads are at stages III and VIII, which are difficult to distinguish otherwise. This parasite can also be used to distinguish virgin-spawning herring from herring that have spawned previously.

The gonads of both spring- and autumn-spawning herring are at the resting stage over a certain period in summer and again in winter. In summer, autumn-spawning herring prepare to spawn and their gonads progress from stage III to stages IV and V (preparatory phase) whilst the gonads of spring spawners are spent and return to stage III from stages VII and VIII (recovery phase). In winter, autumn-spawning herring are emerging from the recovery phase and spring spawners are resting prior to the preparatory phase.

Maximum infection of the testes with oocysts of *E. sardinae* occurs just prior to and during spawning (stages V and VI). The oocysts are shed with the sperm during spawning, and therefore, the prevalence in spent populations drops significantly. Herring populations that have spawned and are in the process of recovering harbor few *E. sardinae* oocyst infections whilst those preparing to spawn show increasing prevalences as they mature. In areas such as the Gulf of St. Lawrence, where both spring- and autumn-spawning herring mix, fish over 4 yr old can be distinguished at the resting stage by the presence or absence of *E. sardinae* oocysts. Uninfected younger adults may be virgin spawners rather than recovering spawners, and in groups consisting of spring and autumn spawners, virgin herring can only be distinguished by age.

The low prevalence found in immature herring is most likely due to the absence of immature fish from spawning aggregations, where the infective oocysts are released. These oocysts are negatively buoyant (pers. obs.), and since they are released with the sperm close to the seabed, dispersal from the spawning grounds can be assumed to be limited. In areas where autumn-spawning herring predominate, such as the Bay of Fundy, this characteristic of restricted dispersal means that prevalence can be used to indicate the percentage of virgin herring entering the spawning stock, e.g. the lower prevalence of *E. sardinae* oocysts in adults spawning in the Bay of Fundy, compared with other areas sampled in autumn (Fig. 18), is a clear example of virgin spawners mixing with herring that have spawned previously.

Discussion

Certain parasites of herring can provide information on various aspects of herring biology. Unfortunately, however, no single parasite species was found that could clearly differentiate one population of herring from another. We concluded that there is extensive mixing of herring in feeding and nursery aggregations. During spawning, when they maintain a relatively discrete population distribution, they do not feed and are, therefore, less likely to acquire a characteristic parasite fauna. Despite this, however, the information provided by the differential prevalences and intensities of infection by certain herring parasites is a valuable contribution to the knowledge already accumulated on herring migrations, feeding, and spawning behavior. Such information is relatively inexpensive to obtain, easy to collate, and derives directly from the natural environment of the herring.

The seven parasite species that emerged from this investigation as potential biological indicators of different aspects of herring biology were from a wide range of parasite groups, and although *A. simplex*, *H. aduncum*, *C. lingua*, and *E. sardinae* fit the criteria for suitable biological "tags" outlined by previous authors (see Kabata 1963; Sindermann 1982), the remaining three species did not. The criteria given for biological indicators by McGladdery (1984) are broader than those of Kabata (1963) and Sindermann (1982) and include species previously considered useless for indicating the biology of their hosts, such as adult digeneans. The only parasite species unsuitable for use as indicator species under the criteria of McGladdery (1984) were those with a uniform, ubiquitous distribution in all herring samples examined, or those that occurred too infrequently to provide an accurate distribution pattern.

The herring movements indicated by the seven parasite species that fulfilled the criteria for a suitable biological indicator showed certain migration routes that had been previously determined using methods such as (i) tagging (Stobo 1983) in the case of movement into and out of the Bay of Fundy and (ii) morphometrics and meristics (Parsons 1972, 1973) in the case of movement into and out of the Gulf of St. Lawrence.

However, certain other herring migrations, based solely on parasite indicators, were previously unrecognized, e.g. the migration from southern Newfoundland offshore, in spring. Parsons and Hodder (1974), however, noted a discrete spring-spawning stock of herring in Fortune Bay over winter that did not appear related to Gulf of St. Lawrence or southwestern Newfoundland stocks.

The use of parasites for indicating the diet of their hosts and distinguishing between different age-groups, as they change from one diet to another, has not previously been recorded. The use of parasites as biological indicators has been restricted primarily to distinguishing between different host populations (e.g. Sindermann 1957b; Margolis 1963; Lubieniecki 1977) or delineating host migrations (e.g. Olson and Pratt 1973; MacKenzie 1975; Hislop and MacKenzie 1976). The value of parasite species for indicating diet changes in herring, however, is limited. As herring increase in age they change to larger prey items gradually and may continue to feed on smaller prey in the absence of larger ones.

The most useful parasite for indicating diet change in the herring examined was *A. simplex*, since it was rarely transmitted to herring under 3 yr old (Fig. 2) and showed a sharp increase in prevalence between herring aged 2 and 4 yr, which was a clear indication of increased consumption of the intermediate, euphausiid hosts. The increase in prevalence in herring over 3 yr old is, undoubtedly, due to cumulative infections and is well documented (Banning and Becker 1978).

The use of parasites for indicating spawning behavior of their fish hosts is also reported for the first time. Previous work on herring spawning groups has been restricted to morphometrics and meristics (Parsons 1972), genetics (Kornfield et al. 1982), and otolith characteristics (Messieh and MacDougall 1984). Since none of these techniques clearly differentiated one spawning group from the other, *E. sardinae* is especially useful for providing supplementary information that can distinguish between the different spawning groups over the periods when the gonads of both spawning groups are at the resting stage of development (late stage VIII to early stage IV).

The use of parasites to indicate recruitment into the adult spawning population has been reported by MacKenzie (1974), who linked juvenile herring from the east coast of Scotland to adults spawning off northwest Scotland, using metacercariae of *Renicola*, and juveniles from the west coast of Denmark to adults caught off the Shetland Islands (North of Scotland), using plerocercoids of *Lacistorhynchus*. The recruitment patterns described by MacKenzie (1974) were, therefore, based on geographic distribution of the parasites. The parasite that appeared useful for indicating recruitment in this investigation, however, was *E. sardinae*, which had a uniform distribution over the localities sampled. The value of *E. sardinae* for indicating recruitment was related to the apparently limited distribution of the infective stages after release on the spawning grounds. Evidence of this was the low prevalence found in the nonspawning fish compared with spawners. The latter pick up infections whilst spawning. The infective oocyst stage of *E. sardinae* can be detected in the maturing testes of the fish, prior to spawning the following year. Herring spawning for the first time would be expected to have low prevalences of *E. sardinae* compared with repeat spawners. Therefore, low prevalences during spawning, such as the one in the Bay of Fundy in autumn (Fig. 18), indicate a large recruitment of virgin spawners into the spawning population. In addition to providing valuable information on different aspects of the biology of herring, these biological indicators have the potential, with continued assessment, to provide information that, when used in conjunction with other data and techniques, could eventually be used to distinguish individual populations of herring.

Acknowledgements

We gratefully acknowledge the assistance of personnel at the Department of Fisheries and Oceans (Scotia–Fundy) Biological Station and the Huntsman Marine Laboratory, St. Andrews, New Brunswick, especially Drs. J. S. Scott, T. D. Iles, and J. Foster. Funding for this project was generously provided by a NSERC grant (A-2581) to M. D. B. Burt, the University of New Brunswick through a GRA to S. E. McGladdery, and a DFO Subvention Grant (09SC. FP 706010C032).

References

ANTHONY, V. C. 1981a. The use of meristic counts in indicating herring stocks in the Gulf of Maine and adjacent waters. Northwest Atl. Fish. Organ. SCR Doc. 81/IX/127, Ser. No. N433: 37 p.

1981b. Status of herring tagging research in the Gulf of Maine. Northwest Atl. Fish. Organ. SCR Doc. 81/IX/133, Ser. No. N439: 3 p.

BANNING, P. VAN, AND H. B. BECKER. 1978. Long term survey data (1965–1972) on the occurrence of *Anisakis* larvae (Nematoda: Ascaridida) in herring Clupea harengus L., from the North Sea. J. Fish Biol. 12: 25–33.

BOYAR, H. C., AND F. E. PERKINS. 1971. The occurrence of a larval nematode (*Anisakis* sp.) in adult herring from ICNAF subareas 4 and 5, 1962–1969. Int. Comm. Northwest Atl. Fish. Res. Doc. 21/99, Ser. No. 257b: 2 p.

GIBSON, D. I. 1972. Flounder parasites as biological tags. J. Fish Biol. 4: 1–9.

GRANT, W. S. 1984. Biochemical population genetics of Atlantic herring, *Clupea harengus*. Copeia 1984: 357–363.

HARE, G. M., AND M. D. B. BURT. 1976. Parasites as potential biological tags of Atlantic salmon (*Salmo salar*) smolts in the Miramichi River system, New Brunswick. J. Fish. Res. Board Can. 33: 1139–1143.

HISLOP, J. R. G., AND K. MACKENZIE. 1976. Population studies of the whiting *Merlangus merlangus* (L) in the northern North Sea. J. Comm. Int. Explor. Mer 37: 98–111.

JONES, R. 1977. Tagging: theoretical methods and practical difficulties, p. 46–66. *In* J. A. Gulland [ed.] Fish population dynamics. Wiley, New York, NY.

KABATA, Z. 1963. Parasites as biological tags. Int. Comm. Northwest Atl. Fish. Spec. Publ. 4: 31–37.

KHAN, R. A., M. DAWE, R. BOWERING, AND R. K. MISRA. 1982. Blood protozoa as an aid for separating stocks of Greenland halibut, *Reinhardtius hippglossoides*, in the northwestern Atlantic. Can. J. Fish. Aquat. Sci. 39: 1317–1322.

KØIE, M. 1979. On the morphology and life-history of *Derogenes varicus* (Müller, 1784) L. 1901 (Trematoda, Hemiuridae). Z. Parasitenkd. 59: 67–78.

KORNFIELD, I., B. D. SIDELL, AND P. S. GAGNON. 1982. Stock definition in Atlantic herring (*Clupea harengus harengus*): genetic evidence for discrete fall and spring spawning populations. Can. J. Fish. Aquat. Sci. 39: 1610–1621.

LOUGH, R. G. 1976. Analysis of various length measurements on larvae collected by the ICNAF larval herring surveys. Int. Comm. Northwest Atl. Fish. Res. Doc. 76/6/58, Ser. No. 3845: 6 p.

LUBIENIECKI, B. 1977. The plerocercus of *Grillotia erinaceus* as a biological tag for haddock *Melanogrammus aeglefinus* in the North Sea and north-east Atlantic. J. Fish Biol. 11: 555–565.

MACKENZIE, K. 1974. The use of parasites in tracing herring recruitment migrations. Int. Comm. Explor. Sea, C.M. 1974/H:31, Pelagic Fish (Northern) Comm. 5 p.

1975. Parasites as indicators of herring migrations in the North Sea and to the north and west of Scotland. Int. Counc. Explor. Sea, C.M. 1975/H:42, Pelagic Fish (Northern) Comm. 7 p.

MACKENZIE, K., AND C. JOHNSON. 1976. Recruitment to the Minch herring population, as determined by the use of parasites as biological tags and a new meristic character. Int. Comm. Explor. Sea, C.M. 1976/H:34, Pelagic Fish (Northern) Comm. 9 p.

MACKENZIE, K., AND S. MEHL. 1984. The cestode parasite *Grillotia angeli* as a biological tag for mackerel in the eastern north Atlantic. Int. Comm. Explor. Sea, C.M. 1984/H:52, Pelagic Fish (Northern) Comm. 7 p.

MARGOLIS, L. 1963. Parasites as indicators of the geographical origin of sockeye salmon, *Oncorhynchus nerka* (Walbaum), occurring in the north

Pacific Ocean and adjacent seas. Int. North Pac. Fish. Comm. Bull. 11: 101–156.

MARGOLIS, L., AND N. P. BOYCE. 1969. Life span, maturation, and growth of two hemiurid trematodes, *Tubulovesicula lindbergi* and *Lecithaster gibbosus*, in Pacific salmon (genus *Oncorhynchus*). J. Fish. Res. Board Can. 26: 893–907.

MARGOLIS, L., G. W. ESCH, J. C. HOLMES, A. M. KURIS, AND G. M. SCHAD. 1982. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). J. Parasitol. 68: 131–133.

MAURER, R. O. 1976. A preliminary analysis of interspecific trophic relationships between the sea herring, *Clupea harengus* Linnaeus, and the Atlantic mackerel, *Scomber scombrus* Linnaeus. Int. Comm. Northwest Atl. Fish. Res. Doc. 76/6/121, Ser. No. 3967: 22 p.

MCGLADDERY, S. E. 1984. Studies of the parasite fauna of Atlantic herring (*Clupea harengus* L.) from the Northwestern Atlantic Ocean. Ph.D. thesis, University of New Brunswick, Fredericton, NB. 286 p.

MESSIEH, S. N., AND C. MACDOUGALL. 1984. Spawning groups of Atlantic herring in the southern Gulf of St. Lawrence. CAFSAC Res. Doc. 84/65: 28 p.

ODENSE, P. H., AND M. C. ANNAND. 1980. Herring (*Clupea harengus*) isoenzyme studies. Int. Counc. Explor. Sea, C.M. 1980/H:25, Pelagic Fish (Northern) Comm.

OLSON, R. E., AND I. PRATT. 1973. Parasites as indicators of English sole (*Parophrys vetulus*) nursery grounds. Trans. Am. Fish. Soc. 102: 405–411.

PANKRATOV, A. M., AND I. K. SIGAJEV. 1974. Studies on Georges Bank herring spawning in 1970. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 125–129.

PARKER, R. R., E. C. BLACK, AND P. A. LARKIN. 1963. Some aspects of fish marking mortality. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 4: 117–122.

PARRISH, B. B., AND A. SAVILLE. 1965. The biology of the Northeast Atlantic herring populations. Oceanogr. Mar. Biol. Annu. Rev. 3: 323–373.

PARSONS, L. S. 1972. Use of meristic characters and a discriminant function for classifying spring- and autumn-spawning Atlantic herring. Int. Comm. Northwest Atl. Fish. Res. Bull. 9: 5–9.

1973. Meristic characteristics of Atlantic herring *Clupea haremgus harengus* L. stocks in Newfoundland, and adjacent waters. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 37–52.

PARSONS, L. S., AND V. M. HODDER. 1974. Some biological characteristics of the Fortune Bay, Newfoundland, herring stock, 1966–71. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 15–22.

PLATT, N. E. 1976. Codworm — a possible biological indicator of the degree of mixing of Greenland and Iceland cod stocks. J. Cons. Int. Explor. Mer. 37: 41–45.

SCOTT, D. M. 1957. Records of the larval *Contracaecum* sp. in 3 species of mysids from the Bras d'Or Lakes, Nova Scotia, Canada. J. Parasitol. 43: 290.

SCOTT, J. S. 1969. Trematode populations in the Atlantic argentine, *Argentina silus*, and their use as biological indicators. J. Fish. Res. Board Can. 26: 879–891.

1975. Geographic variation in incidence of trematode parasites of American plaice (*Hippoglossoides platessoides*) in the Northwest Atlantic. J. Fish. Res. Board Can. 32: 547–550.

1981. Alimentary tract parasites of haddock (*Melanogrammus aeglefinus*) on the Scotian Shelf. Can. J. Zool. 59: 2244–2252.

SHERMAN, K., AND H. C. PERKINS. 1971. Seasonal variation in the food of juvenile herring in coastal waters of Maine. Trans. Am. Fish. Soc. 100: 121–124.

SHERMAN, K., AND J. P. WISE. 1961. Incidence of the cod parasite *Lernaeocera branchialis* in the New England area and its possible use as an indicator of cod populations. Limnol. Oceanogr. 6: 61–67.

SHOTTER, R. A. 1973. Changes in the parasite fauna of whiting (*Odontogadus merlangus*) with age and sex of host, season and from different areas in the vicinity of the Isle of Man. J. Fish Biol. 5: 559–573.

SINDERMANN, C. J. 1957a. Diseases of fishes of the western North Atlantic. V. Parasites as indicators of herring movements. Maine Dep. Sea Shore Fish. Res. Bull. 27: 30 p.

1957b. Diseases of fishes of the western North Atlantic. VI. Geographic discontinuity of myxosporidiosis in immature herring from the Gulf of Maine. Maine Dep. Sea Shore Fish. Res. Bull. 29: 17 p.

1979. Status of Northwest Atlantic herring stocks of concern to the United States. Natl. Mar. Fish. Serv. Tech. Serv. Rep. No. 23: 449 p.

1982. Parasites as natural tags for marine fish: a review. Northwest Atl. Fish. Organ. SCR Doc. 82/IX/90, Ser. No. N599: 18 p.

SMITH, J. W. 1983. Larval *Anisakis simplex* (Rudolphi 1809, det. Krabbe, 1878) and larval *Hysterothylacium* sp. (Nematoda: Ascaridoidea) in euphausiids (Crustacea: Malacostraca) in the North-East Atlantic and northern North Sea. J. Helminthol.57: 167–177.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry: the principles and practice of statistics in biological research. 2nd ed. Freeman and Co., San Francisco, CA. 859 p.

STOBO, W. T. 1983. Report of the "ad hoc" working group on herring tagging. Northwest Atl. Fish. Organ. (Spec. Publ.) SCR Doc. 83/VI/18, Ser. No. N723: 41 p.

STUNKARD, H. W. 1930. The life-history of *Cryptocotyle lingua* (Creplin) with notes on the physiology of the metacercariae. J. Morphol. Physiol. 50: 143–191.

TRUVELLER, C. A. 1971. A study of blood groups in herring (*Clupea harengus*) from the North Sea in connection with the problem of race differentiation. Rap. P.-V. Int. Counc. Explor. Sea 161: 33–39.

WARING, G. T. 1981. Results of the International herring tagging programme conducted by USA in the Gulf of Maine, Georges Bank and contiguous waters from 1976–78. Northwest Atl. Fish. Organ. SCR Doc. 81/IV/122, Ser. No. N428: 24 p.

Variabilité interannuelle d'un indice d'intensité des remontées d'eau dans le secteur du cap Blanc (Mauritanie)

Robert Arfi

Centre d'Océanologie de Marseille URA-41, Faculté des Sciences de Luminy, case 901, F 13288 Marseille Cedex 9, France

Arfi, R. 1985. Variabilité interannuelle d'un indice d'intensité des remontées d'eau dans le secteur du cap Blanc (Mauritanie). Can. J. Fish. Aquat. Sci. 42: 1969–1978.

Les eaux côtières bordant le Sahara atlantique sont concernées par des phénomènes hydrodynamiques (upwellings, alternances de masses d'eau) particulièrement intenses, qui influent profondément sur leur productivité. Les remontées d'eau sont liées au régime des vents, où prédominent à longueur d'année les alizés du nord-est. L'étude d'une série chronologique de ces vents portant sur une période de 28 a permet de montrer que, loin de présenter la régularité et la constance qu'on lui prête, le régime des alizés a montré d'importantes variations au cours de cette période. Corrélativement, l'upwelling se développant au large de ces côtes a connu d'amples fluctuations d'intensité : à une période de forte activité (1955–1960) a succédé une période d'intensité minimale (1961–1969), suivie d'années très irrégulières (1970–1982), mais montrant un retour progressif aux conditions premières. Ces variations importantes, qui engendrent de profondes conséquences hydrologiques et agissent sur les potentialités trophiques du milieu, peuvent être rapprochées des anomalies biologiques relevées au cours de cette même période dans les pêcheries du Nord-Ouest africain.

The coastal waters along the Atlantic Sahara are characterized by intense hydrodynamic phenomena (upwellings, water mass alternations) throughout the year that deeply influence their productivity. Deep-water arrivals at surface levels are closely related to the local prevailing winds dominating all year long, the Northeast Trade winds. Working on a time series of 28 yr shows that far from being regular and constant during that period, the Trade winds were very variable. The coastal upwellings induced by these winds showed important intensity variations: after years of strong activity (1955–60) there was a period of minimal activity (1961–69), which was followed by a period of great irregularity (1970–82), showing a progressive return to the initial conditions. These important variations inducing deep hydrological consequences and modifications of water trophic potentialities can be related to the biological anomalies observed during the same time in the Northwest Africa fisheries.

Reçu le 30 août 1984
Accepté le 23 juillet 1985
(J7930)

Received August 30, 1984
Accepted July 23, 1985

Les eaux côtières bordant le nord-ouest du continent africain sont concernées par des phénomènes hydrodynamiques très intenses (upwelling, alternance saisonnières de masses d'eau) qui retentissent spectaculairement sur leur productivité (Minas *et al.* 1982). Au nord de cet ensemble qui s'étend du Maroc à la Guinée, le littoral saharien compris entre les caps Barbas et Timiris (fig. 1) occupe une place privilégiée (Wooster *et al.* 1976) : secteur charnière, il est intéressé par d'importantes remontées d'eau, favorisées par la rectitude de la côte et la proximité de canyons ou fosses sous-marines. Ces remontées sont bien entendu liées aux particularités du régime du vent prédominant dans ce secteur géographique, où règnent avec force et permanence les alizés du nord-est. On observe également dans cette région l'alternance saisonnière des eaux centrales sud atlantiques (ECSA, parfois dénommées « guinéennes »), plus chaudes et relativement salées, et des eaux centrales nord atlantiques (ECNA, aussi appelées « canariennes »), plus froides et relativement plus salées (Sverdrup *et al.* 1942; Tomczak 1977). Globalement, ces phénomènes hydroclimatologiques dépendent du déplacement de la position du front intertropical (FIT) au cours de l'année. De nombreux travaux, d'abord généraux (Furnestin 1959; Berrit 1961, 1962; Longhurst 1962; Postel 1968; Berrit 1973), puis plus localisés (Jones 1972; Fraga 1974; Mittelstaedt *et al.* 1975; Pillsbury *et al.* 1975; Schemainda *et al.* 1975; Wooster *et al.* 1976; Barton *et al.* 1977; Speth *et al.* 1978; Sedykh 1979; Speth et Khone 1983), ont précisé les grandes lignes de l'hydroclimat au large du Sahara Atlantique, ou décrit les principaux phénomènes hydrodynamiques liés aux remontées d'eau (Mittelstaedt 1983).

Peu d'entre eux ont cependant abordé la variabilité interannuelle de ces phénomènes, faute de pouvoir disposer de données du temps (et en particulier des vents) sur des périodes suffisamment longues. On ne sait donc pas si le régime du vent, supposé particulièrement régulier, présente la constance qu'on lui prête, ou si, au contraire, il montre d'importantes variations d'une année à l'autre. Corrélativement, l'upwelling se développant au large de ces côtes connaît-il une régularité relative ou présente-t-il au contraire d'importantes fluctuations d'intensité au cours du temps? Les réponses à ces questions, tout en permettant une meilleure connaissance générale du cycle hydroclimatique, pourraient fournir une appréciation plus fine de la variabilité temporelle au moins à l'échelle mensuelle, et contribuer à une gestion plus rationnelle des stocks halieutiques par une approche plus écologique du système. On ne peut, en effet, dissocier le poisson (en particulier le migrateur) de son environnement, surtout si ce dernier contrôle les comportements liés à la

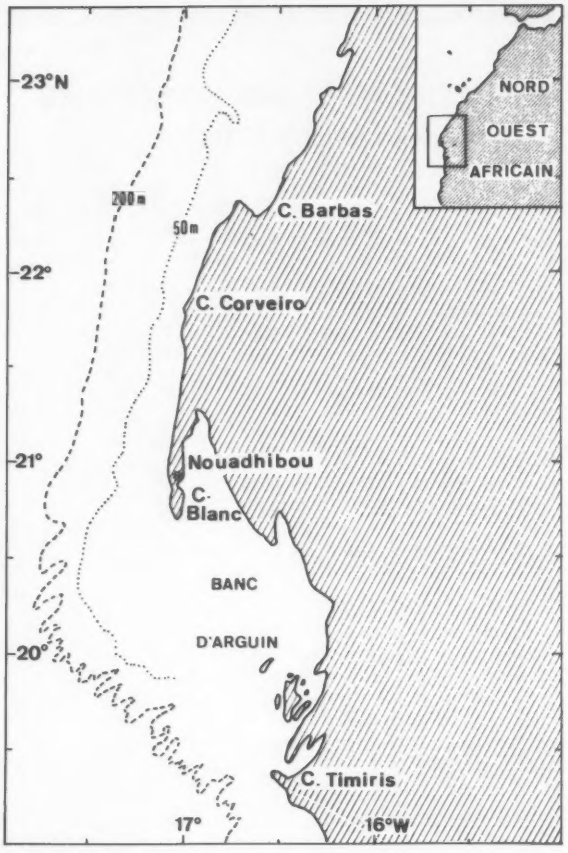


FIG. 1. Carte du littoral atlantique saharien entre les caps Barbas et Timiris, et emplacement (*) du point de mesure des variables météorologiques.

reproduction et à la nutrition (et donc à la productivité potentielle du milieu).

Un traitement des données météorologiques (vents) collectées en un point du littoral nord-mauritanien (aéroport de Nouadhibou, 20° 55′ N, 17° 02′ W) permet de s'intéresser à ce problème. Pour Stuart *et al.* (1975), les vents mesurés à terre le long du littoral du cap Blanc reflètent assez fidèlement la situation en mer, telle que déterminée à partir de mesures obtenues par des capteurs sur bouées, et ce, pour une bonne partie du littoral du Sahara atlantique. La période couverte par ce travail s'étend de 1955 à 1982 et a été choisie afin de concorder avec une série de données hydrologiques étudiées par ailleurs. À partir des vents moyens mensuels, caractérisés en direction et en vitesse, un indice d'upwelling moyen mensuel a pu être calculé. L'approche descriptive et statistique de cette série chronologique amène une discussion sur les liaisons possibles de ces éléments avec le régime hydrologique local, comme avec les anomalies biologiques observées au cours de cette période.

Matériel et méthodes

L'ensemble des données météorologiques a été mis à notre disposition par le bureau de l'ASECNA (Agence pour la Sécurité de la Navigation Aérienne) de Nouadhibou (R.I. de Mauritanie). Pour la période s'étendant de janvier 1955 à décembre 1982, l'occurrence mensuelle (exprimée en jours) des vents sur cinq secteurs géographiques prédominants (N à NE, NE à SE, SE à SW, SW à NW et NW à N) et des vents nuls a été relevée, ainsi que la vitesse moyenne mensuelle de la composante du vent parallèle à la côte. Les résultats exploités portent sur la direction et la vitesse du vent observée 8 fois par jour à la station météorologique. Le regroupement de ces observations au sein des différentes directions géographiques d'où soufflent les vents permet de calculer le nombre d'heures (et donc de jours) par mois où tel régime du vent était noté. Ces données peuvent également être exprimées en fréquence sur un mois, une année ou plus. À partir de ces résultats, on calcule le transport d'Ekman mensuel perpendiculaire à la côte (Wooster *et al.* 1976) selon la formule suivante :

$$M = \frac{\tau}{f}$$

pour les régimes des vents de nord, soufflant parallèlement à la côte (la péninsule du cap Blanc est orientée NNE à SSW, comme les isobathes du plateau continental).

Dans ce rapport, f est le facteur de Coriolis à la latitude de Nouadhibou, τ est la composante de la tension du vent parallèle à la côte, de la forme $\rho \cdot c \cdot v^2$ (ρ est la densité de l'air, c correspond à un coefficient fixé à $1{,}25 \cdot 10^{-3}$, d'après Hidy (1972), et v est la composante de la vitesse du vent parallèle à la côte). La valeur de M sera donc nulle pour les régimes des vents de sud, soufflant aussi parallèlement à la côte, mais dans un sens défavorable aux remontées d'eau plus profondes. Pour rendre les résultats comparables à ceux obtenus par d'autres auteurs, les valeurs ont été ramenées en $m^3 \cdot s^{-1}$ par 10 m linéaires de côte. On dispose donc d'une chronique de la variation annuelle du transport d'Ekman face à la péninsule du cap Blanc, ce qui permet de déceler d'éventuelles fluctuations entre des périodes aux caractéristiques différentes. L'emploi de cet indice correspondant à un modèle simple et idéalisé s'explique par la relative simplicité et par la permanence du régime des vents observé dans ce secteur, la force de ces vents comme la quasi absence de vent de sud ou de sud-ouest. Pour Halpern (1977), cette expression s'accorde assez bien aux fluctuations rapides du transport vers le large calculé à partir de mesures des courants locaux.

Résultats

Régimes de vent affectant le littoral saharien entre 15 et 25° N

Le littoral saharien atlantique est inclus géographiquement dans le secteur d'oscillation annuelle du système anticyclonique de l'Atlantique Nord. Tout au long de l'année, on observe une zone de hautes pressions au voisinage des Açores. En hiver, elle esta centrée entre les archipels des Canaries et des Açores; vers le sud, les pressions diminuent progressivement jusqu'à la zone des basses pressions équatoriales. En été, le système anticyclonique gagne vers le nord et l'ouest, tout en se renforçant. Simultanément, les basses pressions s'étendent jusque vers 15° N et envahissent progressivement le Sahara central, tout en se creusant. L'oscillation annuelle se traduit par un mouvement du complexe atmosphérique du sud vers le nord de février à août, et du nord vers le sud de septembre à janvier.

L'existence de ce gradient de pression atmosphérique et le déplacement saisonnier de son centre d'action sont à l'origine

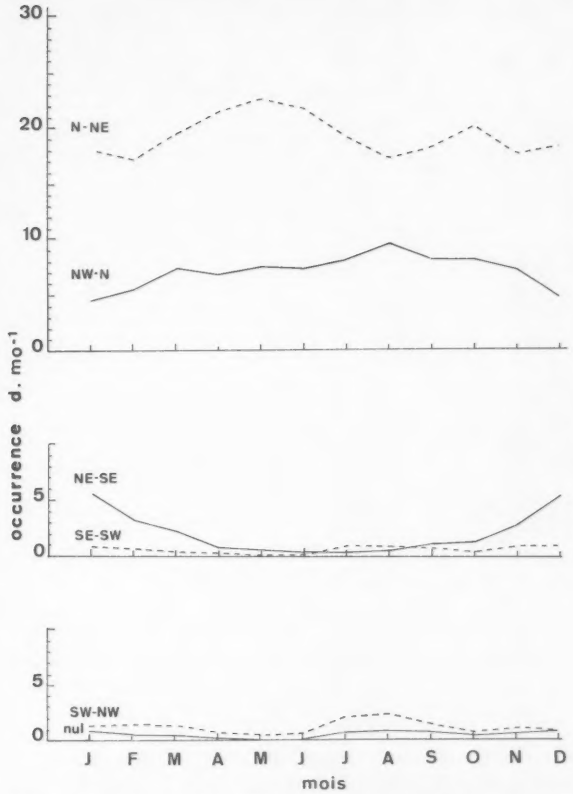


FIG. 2. Occurrences des différents types de vent et de vent nul observés à Nouadhibou. Valeurs mensuelles moyennes portant sur la période 1955–1982.

des vents affectant le littoral saharien entre 15 et 25°N : les alizés du nord-est. La principale caractéristique de ces vents est leur régularité, tant en force qu'en fréquence. Dans la région de Nouadhibou, on les observe tout au long de l'année, prédominant très largement sur les autres types de vent : 63,1 % (soit 230 jours par an) pour les vents de N–NE, et 23 % (soit 84 jours par an) pour ceux de NW–N, mais seulement 6,6 % (24 jours par an) pour les vents de NE–SE, 1,7 % (6 jours par an) pour les vents de SE–SW, et 3,7 % (14 jours par an) pour les vents de SW–NW. Quant aux jours sans vent, on n'en observe que 7 par an, soit 1,9 % des observations annuelles (moyennes sur la période 1955–1982). La vitesse moyenne des alizés est relativement élevée (entre 6 et 8 $m \cdot s^{-1}$), et des pointes supérieures à 15 $m \cdot s^{-1}$ sont très fréquentes. D'octobre à mars, on peut observer de courtes périodes de vents du secteur est, parfois violents (l'Harmattan), et plus rarement, des périodes de vents d'ouest ou de sud. Les périodes de calme (vitesse de vent inférieure ou égale à 1 $m \cdot s^{-1}$) sont peu nombreuses et rarement de longue durée (fig. 2). Au sud du cap Timiris, le régime des alizés ne prédomine plus, tant en fréquence qu'en vitesse, et tend à être remplaçé par des vents du secteur est (l'Harmattan y est plus sensible qu'à Nouadhibou) et d'ouest, annonçant la proximité de la zone soumise au régime de la mousson du sud-ouest. Les périodes de vent faible ou nul sont beaucoup plus importantes, et d'une manière générale, la force du vent est beaucoup plus faible que dans le nord du pays.

Série météorologique observée à Nouadhibou entre 1955 et 1982

Le secteur d'étude est réputé pour la régularité du régime d'alizés du NE qui y prédomine (Anonyme 1959). Or, la période 1955–1982 présente, associée à cette prédominance, une certaine irrégularité du régime des vents, comme le montre la série chronologique des occurrences mensuelles des vents dominants (fig. 3). On y remarque l'alternance de périodes où les alizés de N–NE prédominent nettement (entre 60 et 80 % des situations) et d'autres où ils représentent un pourcentage moins élevé (entre 30 et 50 %). Dans ce dernier cas, le régime des vents est plus diversifié. Une autre caractéristique de ces deux types météorologiques se rapporte au nombre d'observations de vent nul : il est très faible lorsque le vent est nettement orienté au secteur N–NE, mais augmente franchement lorsque le vent de NW–N s'installe durablement. En moyenne sur 28 ans, une année type du régime des alizés présente deux périodes nettement individualisées (fig. 4) :

1) lors de la première période, la vitesse moyenne du vent de secteur N–NE augmente régulièrement à partir de janvier pour atteindre son maximum au mois de juin; au cours de ce premier semestre, le nombre moyen de jours d'observation du vent de N–NE tend à croître et devient maximum au mois de mai.
2) au cours de la seconde période (juillet à décembre), la vitesse moyenne de ce vent diminue progressivement, tandis que les observations mensuelles diminuent, elles, de manière plus irrégulière.

Mais entre les années 1955 et 1982, trois périodes nettement différenciées du point de vue de leurs caractéristiques climatologiques se sont succédées (fig. 5 et tableau 1) :

1) De 1955 à 1961, avec des alizés du NE forts et réguliers; ils dominent largement (en moyenne 72,3 % des observations) et présentent une évolution annuelle « classique » : renforcement progressif de leur occurrence mensuelle et de leur vitesse moyenne de février à mai, puis diminution tout aussi progressive de ces caractères au cours de l'été, avec une augmentation relative en automne; janvier montre la particularité d'avoir de nombreux jours de vents de N–NE mais avec une vitesse moyenne minimale. Ces années sont également caractérisées par la faiblesse des observations de vent nul (0,5 jour par mois, en moyenne).
2) De 1962 à 1969, période présentant un cycle annuel aux amples variations mensuelles (fig. 6), où les alizés du NE ne prédominent plus aussi nettement (49 % des observations, en moyenne), mais coexistent avec un régime plus diversifié où les vents de NW–N, relativement réguliers, prennent parfois une grande importance (34,9 % des observations, en moyenne). Les vitesses des vents sont nettement plus faibles, mais l'allure du cycle moyen reste inchangée par raport à celle de la période précédente. Les occurrences de vent nul sont, par contre, en sensible augmentation (3,3 jours par mois, en moyenne).
3) De 1970 à 1982, où l'on retrouve globalement les conditions prévalant au cours de la première période (les vents de N–NE représentent en effet 66,2 % des situations). Mais si les occurrences mensuelles moyennes des vents de N–NE demeurent inférieures à celles observées de 1955 à 1961, leurs vitesses sont, en moyenne, plus élevées au cours de cette troisième période, particulièrement au cours du dernier trimestre de ces années. Le régime de NW–N est plus irrégulier (18,5 % des observations, que l'on peut

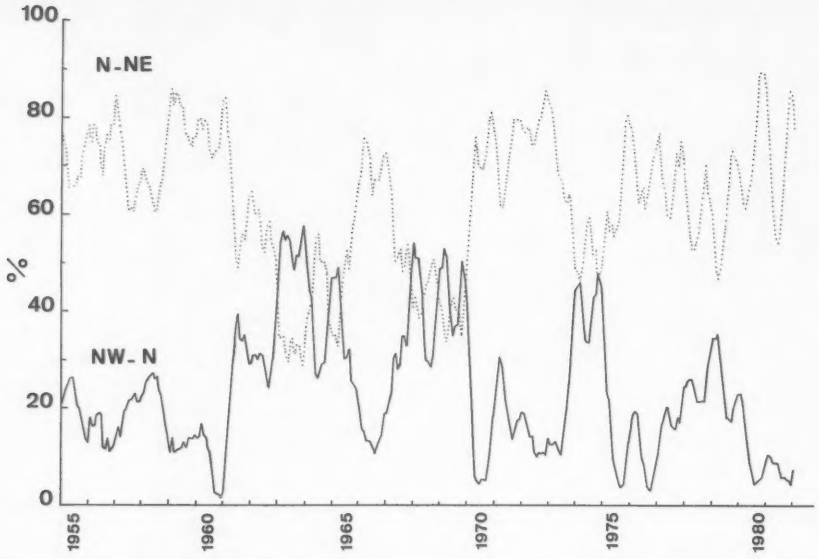


FIG. 3. Moyenne mobile d'ordre 6 des fréquences d'occurrence mensuelle des vents des secteurs N–NE (···) et NW–N (—) observés à Nouadhibou.

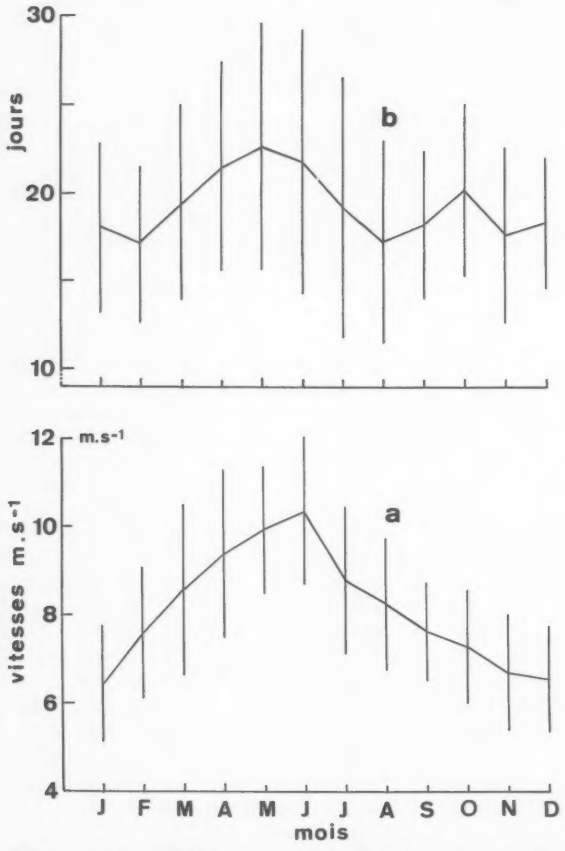


FIG. 4. Cycle annuel des vitesses moyennes (a) des vents de N–NE et (b) de leurs occurrences mensuelles à Nouadhibou entre 1955 et 1982. De part et d'autre du point moyen est portée la valeur de l'écart type.

comparer aux 17,7 % de la période 1955–1961), mais il peut parfois présenter une importance comparable à celle qu'il avait au cours de la période précédente, en particulier en 1973 et en 1974 : par leurs caractéristiques, ces deux années se rapprochent beaucoup de celles de 1962 à 1969. Le changement climatologique qu'a connu le secteur étudié sur la période considérée a donc concerné toutes les caractéristiques du régime des alizés de N–NE :

a) la fréquence d'observation à l'échelle de l'année :
   i) 1955–1961 : 22 jours par mois, en moyenne
   ii) 1962–1969 : 15 jours par mois, en moyenne
   iii) 1970–1982 : 20 jours par mois, en moyenne;
b) l'amplitude du cycle annuel, beaucoup moins marquée au cours de la seconde période qu'au cours des deux autres : les pics saisonniers (mai et octobre) sont toujours présents, mais beaucoup moins accentués entre 1962 et 1969;
c) la variabilité mensuelle du régime des vents (écarts types), minimale au cours de la période 1955–1961 et maximale de 1962 à 1969, tend à diminuer entre 1970 et 1982;
d) l'allure du cycle annuel des vitesses des vents, inchangée au cours des trois périodes, mais avec de très nettes différences quantitatives.

Le régime du vent dominant dans la région de Nouadhibou a donc connu de profonds changements tant qualitatifs que quantitatifs entre 1955 et 1982. Ces variations en direction et en vitesse auront de profondes répercussions sur la durée et la puissance des upwellings côtiers dans le secteur de la péninsule du cap Blanc, pourtant réputés pour leur régularité tout au long de l'année (Schemainda et Nehring 1975). En effet, si un régime de N–NE avec des vents plutôt forts favorise l'apparition et l'installation durable d'un phénomène de remontée d'eau, en soufflant parallèlement à la côte et aux isobathes au large de la péninsule, il n'en va pas de même pour un régime de NW–N dominant. Leur efficacité à entretenir un upwelling serait en effet moindre, plus par leur irrégularité et leur vitesse plus faible qu'en raison de la direction d'où ils soufflent.

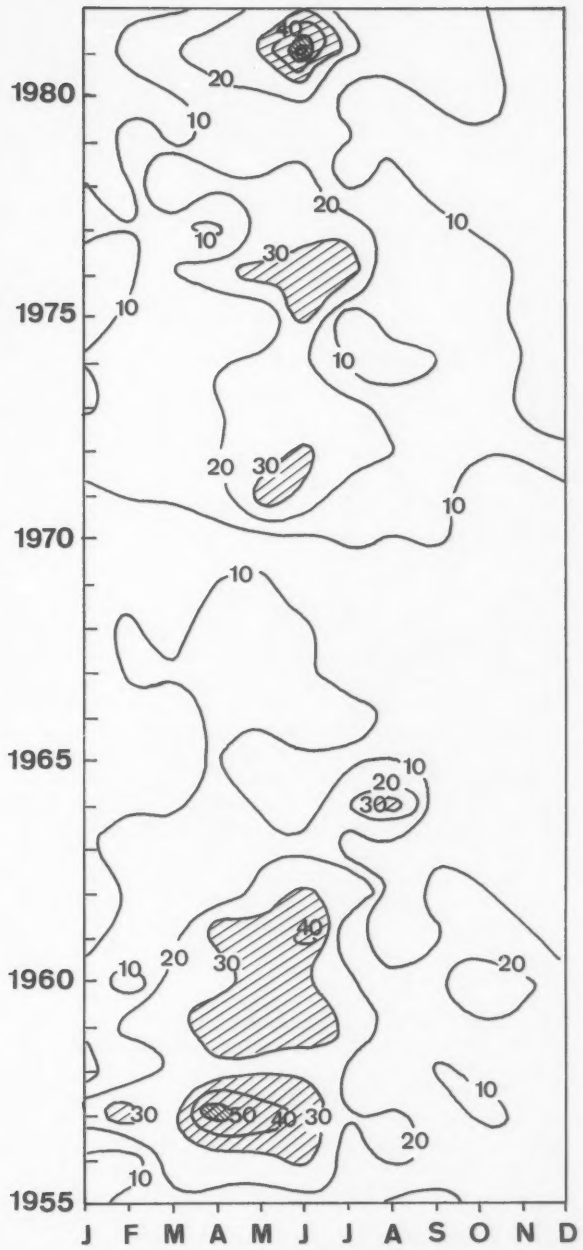


FIG. 5. Variations mensuelles et annuelles (période 1955–1982) du transport d'Ekman à la latitude de Nouadhibou. Les valeurs sont exprimées en $m^3 \cdot s^{-1}$ par 10 m de littoral; les surfaces hachurées correspondent aux valeurs supérieures ou égales à $30\ m^3 \cdot s^{-1}$ par 10 m de littoral.

Régime de vent et transport d'Ekman dans le secteur de Nouadhibou entre 1955 et 1982

Les vents du secteur nord, dominants sur cette partie du littoral de la Mauritanie, favorisent globalement les phénomènes de remontée d'eau. Cependant, leur irrégularité à long terme laisse supposer un régime d'upwelling largement variable, avec

TABLEAU 1. Fréquence mensuelle et nombre de jours par an d'occurrence de vent nul et de vents des secteurs N–NE et NW–N observés au cours des périodes 1955–1961, 1962–1969 et 1970–1982. (Les valeurs entre parenthèses correspondent aux écarts types des moyennes.)

	1955–1961	1962–1969	1970–1982
Fréquence mensuelle			
Vent nul	0,5 (0,7)	3,3 (4,1)	1,8 (2,2)
Secteur N–NE	72,3 (14,9)	49,9 (18,1)	66,2 (17,3)
Secteur NW–N	17,7 (11,3)	34,9 (17,6)	18,5 (15)
Nombre de jours par an			
Vent nul	2	12	7
Secteur N–NE	264	182	242
Secteur NW–N	65	127	68

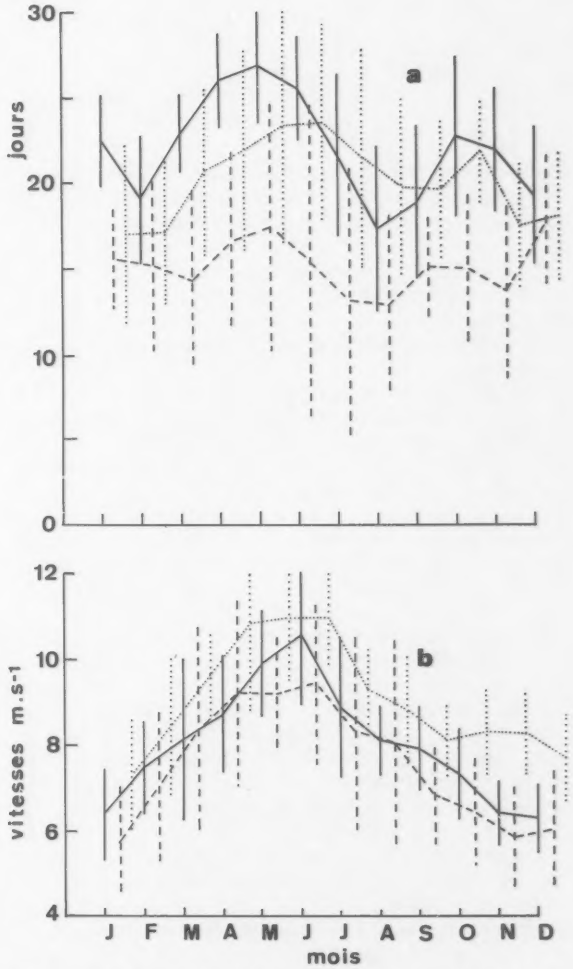


FIG. 6. Cycle annuel moyen des occurrences mensuelles (a) des vents de secteur N–NE et (b) de leurs vitesses à Nouadhibou entre 1955 et 1961 (—), 1962 et 1969 (---), et 1970 et 1982 (···). De part et d'autre du point moyen est portée la valeur de l'écart type. Les courbes sont progressivement décalées vers la droite par souci de clarté de la figure.

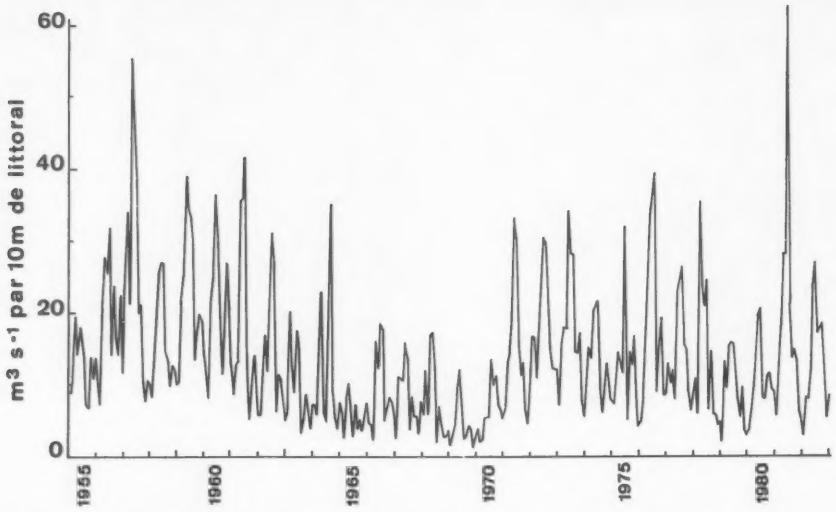


FIG. 7. Transport d'Ekman : chronique mensuelle (période 1955–1982) reflétant les variations d'intensité de cet indice de l'upwelling nord-mauritanien à la latitude de Nouadhibou.

d'importantes fluctuations interannuelles, au moins sur la période d'étude. L'intensité mensuelle du transport d'Ekman est directement proportionnelle à la vitesse et à l'occurrence de la composante du vent parallèle à la côte. Sur un cycle annuel moyen, la fréquence des vents de N–NE varie assez peu d'un mois à l'autre; par contre, la vitesse moyenne varie dans des proportions importantes et de manière cyclique. Au large de Nouadhibou, les upwellings présenteront donc également une évolution saisonnière, voyant leur activité croître de février à juin et décroître de juillet à octobre; avec des vitesses faibles, les mois les plus froids sont ceux où l'activité hydrodynamique engendrée par les vents serait la moins importante.

Cette rythmicité se retrouve sur la chronique des volumes d'eau transportés mensuellement entre 1955 et 1982 (fig. 7). Sur une année, les différences peuvent être considérables d'une saison à l'autre, même si, à la latitude du cap Blanc, le phénomène de remontée paraît être présent tout au long de l'année, ce qui n'est pas le cas plus au nord — côtes du Rio del Oro — ou plus au sud — côtes sénégalo-guinéennes — où il est plus saisonnier (Schemainda et Nehring 1975). Dans le secteur du cap Blanc, l'upwelling est maximal en mai et en juin et moins actif de novembre à février. Il faut cependant noter que la période d'intensité maximale peut s'observer de mars à août et la période d'intensité minimale de juillet à mars. Enfin, activités maximales et minimales peuvent se succéder très rapidement : les volumes transportés mensuellement au large de la péninsule du cap Blanc varient dans des proportions très importantes, au sein d'un cycle annuel nettement marqué.

Les cycles annuels moyens correspondant aux trois types de conditions météorologiques définis plus haut vont, eux, différer de manière sensible (fig. 8) :

1) les années 1955 à 1961 présentent les intensités maximales du transport, avec un pic principal (avril à juin) et un pic secondaire (octobre). L'activité générale de l'upwelling croît au début de cette période, puis décroît rapidement à partir de 1960; le transport d'Ekman annuel estimé est compris entre 400×10^6 et 800×10^6 $m^3 \cdot an^{-1}$ par 10 m de littoral.
2) les années 1962 à 1969 montrent un très fort ralentissement de l'activité quel que soit le mois considéré, mais l'allure générale du cycle annuel reste semblable à celle de la période précédente. Les valeurs du transport annuel sont comprises entre 150×10^6 et 400×10^6 $m^3 \cdot an^{-1}$ par 10 m de côte; elles décroissent globalement au cours de ces années, pour atteindre un minimum en 1969.
3) enfin, les années 1970 à 1982 présentent un cycle annuel assez proche de celui de la période 1955–1961, mais un seul pic (juin) est nettement marqué, et les valeurs demeurent plus faibles tout au long de l'année moyenne. L'activité globale augmente par rapport à la période précédente (valeurs annuelles comprises entre 220×10^6 et 640×10^6 $m^3 \cdot an^{-1}$ par 10 m de côte), mais elle montre de fortes fluctuations d'une année à l'autre.

Discussion

Soumis aux variations interannuelles des vents qui les engendrent, les phénomènes d'upwelling ont présenté d'importantes fluctuations au cours de la période d'étude. Maximale à la fin des années 50, l'intensité du transport d'Ekman a décru progressivement pour être minimale une dizaine d'années plus tard. La dernière décennie montre un retour irrégulier aux conditions premières, retour qui s'accompagne d'amples variations d'une année à l'autre. Les conséquences de ce processus sur les conditions hydrologiques vont concerner les caractéristiques thermiques et trophiques de ces eaux :

1) le phénomène d'upwelling amène en surface des eaux profondes, donc froides; toute variation de son intensité va s'accompagner de sensibles modifications des conditions thermiques locales. En cas de nette diminution de l'activité de remontée, les eaux de la couche superficielle seront moins froides, en particulier en hiver; de même, au cours des mois d'été, caractérisés dans ce secteur par la progression vers le nord d'eau centrale sud atlantique (ECSA) (Tomczak 1978), les diminutions de température causées par l'irruption de ces eaux plus profondes seront moins marquées et moins fréquentes. Inversement, si l'intensité de l'upwelling augmente, la couche plus superficielle sera

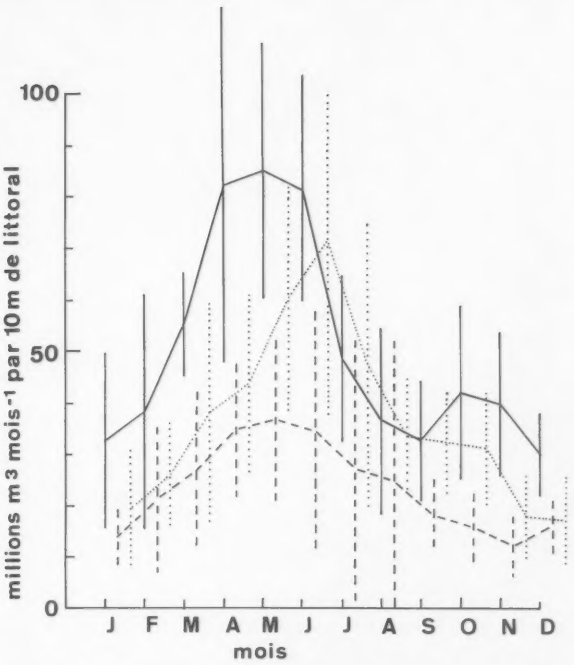


FIG. 8. Cycle annuel moyen du transport d'Ekman à la latitude de Nouadhibou entre 1955 et 1961 (—), 1962 et 1969 (---) et 1970 et 1982 (···). De part et d'autre du point moyen est portée la valeur de l'écart type. Les courbes sont progressivement décalées vers la droite par souci de clarté de la figure.

plus froide (le phénomène sera accentué en hiver), et le réchauffement des eaux de surface causé par l'envahissement estival des eaux de type ECSA sera moins marqué. Considérant l'alternance saisonnière ECNA (eaux froides, relativement plus salées — hiver) / ECSA (eaux chaudes, relativement moins salées — été), comme étant la situation de base (Sverdrup *et al.* 1942) à laquelle se surimpose une variabilité à plus haute fréquence liée à l'intensité et à la relative irrégularité du régime des vents, il est possible d'opposer les deux situations suivantes :

a) en cas d'intensification de l'activité de l'upwelling — et donc du régime des vents du secteur N–NE — les caractéristiques thermiques de type ECNA seraient accentuées en saison froide, avec des eaux superficielles encore plus froides, comme en saison chaude, où la persistance des alizés entraverait la progression du sud vers le nord des eaux « guinéennes » et favoriserait la persistance des eaux « canariennes » au large du secteur étudié.

b) en cas de diminution de cette activité, les caractéristiques thermiques de type ECSA seraient accentuées en été (moindre occurrence des situations d'upwelling et facilitation de l'envahissement du littoral nord-mauritanien par les eaux « guinéennes »), tandis que le caractère ECNA ne serait pas favorisé au cours de l'hiver. Tous les cas de figure intermédiaires seraient en outre possibles lorsque l'on sera en période d'augmentation ou au contraire de diminution de l'intensité des phénomènes de remontée d'eau plus profonde.

2) les eaux concernées par des phénomènes d'upwelling présentent souvent des teneurs élevées en sels nutritifs et provoquent l'enrichissement des couches superficielles appauvries. Ce schéma peut cependant s'avérer faux quand les eaux plus profondes sont moins riches que les eaux de surface avec lesquelles elles se mélangent ou qu'elles chassent vers le large lors de leur installation. Cela peut être le cas dans la région nord-mauritanienne où une alternance saisonnière oppose les eaux centrales nord atlantiques (ECNA) aux eaux centrales sud atlantiques (ECSA) : ces dernières présentent en effet des teneurs en nitrate et en phosphate bien supérieures à celles des premières (Fraga 1974), et ce, dans un rapport de 1 à 3. Selon la force du vent, sa direction, et la durée de « l'évènement » météorologique (favorisant plus ou moins une remontée d'eau), mais aussi la saison (et donc le type de masse d'eau remontée, plus ou moins riche en éléments nutritifs), la productivité potentielle résultante dans le secteur sera donc très différente. Une activité limitée de l'upwelling favorisant en outre l'installation et la persistance des eaux de types ECSA (donc plus riches que les eaux de type ECNA avec lesquelles elles alternent saisonnièrement), l'enrichissement global résultant comme les potentialités trophiques seront donc, paradoxalement, plus importantes. Enfin, une diminution relative de la force du vent ou l'alternance de situations de remontée d'eau intense et de situations sans remontée d'eau peuvent s'avérer être des facteurs favorisant la productivité primaire (Huntsman et Barber 1977; Jones et Halpern 1981; Small et Menzies 1981). Ce schéma pourrait s'appliquer aux années où les alizés de N–NE ne prédominent pas sur les vents de secteur NW–N.

Ignorée ou mal connue, la variabilité interannuelle de l'intensité de l'upwelling nord-mauritanien ne pouvait être prise en compte par les principaux travaux océanographiques réalisés dans ce secteur (programmes CINECA et Joint en particulier). L'ampleur de ces fluctuations laisse penser que les conclusions auxquelles parviennent les différents auteurs correspondent plus particulièrement à des conditions d'upwelling relativement actif (campagne du printemps 1971) ou modéré (campagne du printemps 1974). Observée sur la chronique météorologique détaillée obtenue au cours de l'opération Joint I (Stuart *et al.* 1975), la succession de régimes du vent favorables puis défavorables aux remontées d'eau a été retenue comme régime de base pour cette partie du Nord-Ouest africain (Barton *et al.* 1975; Coste et Minas 1982). Cette caractéristique est également proposée par Codispoti *et al.* (1982) pour opposer les régimes des upwellings du Pérou vers les 15° S (les vents défavorables aux remontées sont « extrêmement rares ») et de l'Ouest africain vers les 21° N (les périodes de vents défavorables aux remontées y surviendraient avec régularité). Or, dans le secteur du cap Blanc, 1974 est une année où les alizés de N–NE ne prédominent pas, mais alternent avec des vents de NW–N, aux vitesses nettement plus faibles : le schéma hydroclimatologique proposé par Codispoti *et al.* (1982) correspond donc à un régime d'upwelling peu intense, aux caractéristiques proches de celles de la période 1962–1969 et donc très différentes des conditions de forte remontée (période 1955 à 1961, ou certaines années de la période 1970 à 1982), au cours desquelles les alternances de régime de vent sont beaucoup plus limitées, voire absentes lorsque les alizés de N–NE représentent près de 100 % des observations mensuelles à Nouadhibou. Le régime général de l'upwelling nord-mauritanien peut donc s'avérer être très proche de celui du Pérou, par exemple, lorsque les vents de N–NE,

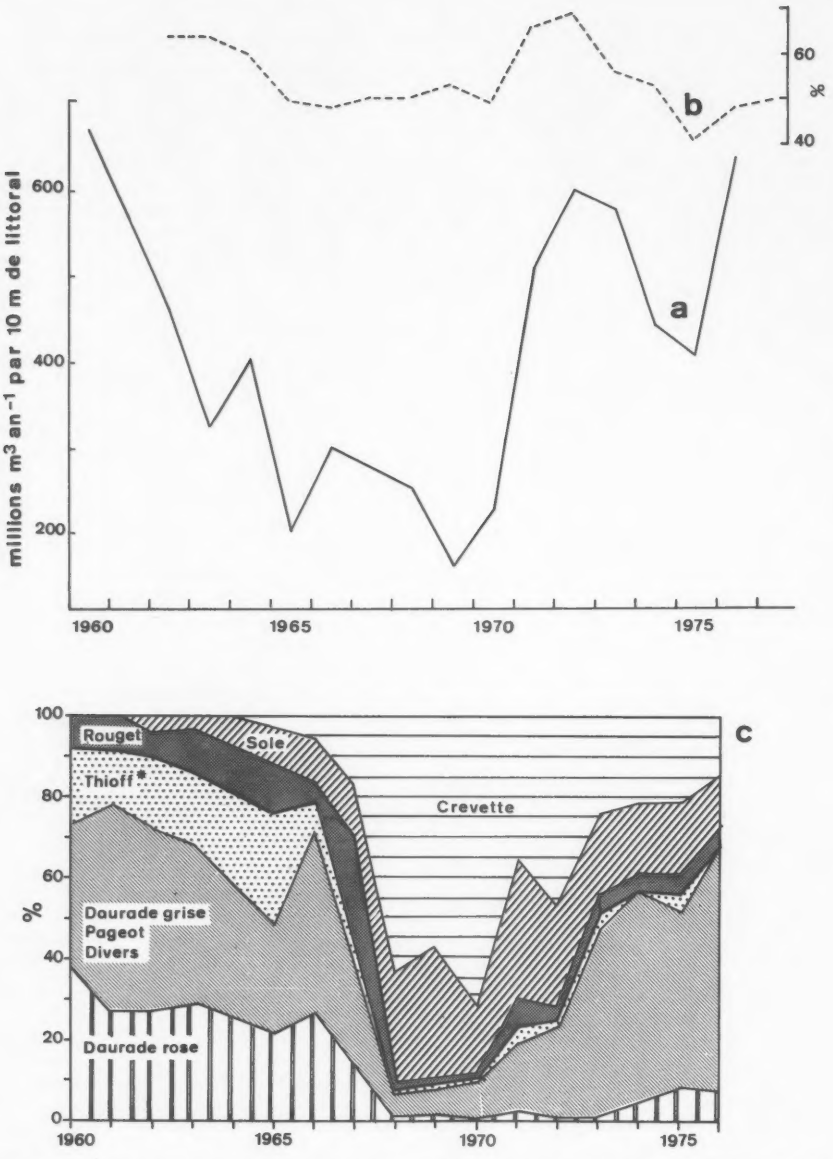


FIG. 9. (a) Évolution du transport annuel d'Ekman (période 1960–1977) à la latitude du cap Blanc, (b) captures des sardiniers dakarois (pourcentage de *Sardinella aurita* par rapport aux captures de petits pélagiques) d'après Freon *et al.* (1978) et (c) évolution de la composition spécifique des débarquements à Dakar (Sénégal), en pourcentage des principales catégories commerciales, d'après Garcia *et al.* (1978).

forts et réguliers, prédominent; il peut en différer significativement en cas d'alternance des alizés de N–NE avec un régime de NW–N, vents faibles et irréguliers dans ce secteur.

Ces changements importants sont demeurés insoupçonnés, faute de pouvoir disposer de séries suffisamment longues. Or, les possibilités de variation de l'activité de remontée, et donc de l'enrichissement des eaux, existent puisque les séquences climatologiques ont nettement changé de caractère au cours de la période étudiée. La séquence hydrologique qui débute en 1970 fait suite à une période aux caractéristiques météorologiques différentes et correspond à la période de sècheresse continentale centrée autour de 1973; en mer, de nombreux phénomène biologiques apparus à cette même époque peuvent être rapprochés de ces modifications de l'hydroclimat. Au cours de cette période, les eaux côtières du nord-ouest africain ont présenté des anomalies que l'on peut rapprocher des modifications écologiques entrainées par les variations climatiques. L'un de ces importants phénomènes a été la prolifération du baliste (*Balistes carolinensis*) le long des côtes de l'Ouest africain (Caveriviere 1982) : cette espèce pélagique, considérée comme

rare ou au moins peu abondante au début des années 70 (Williams 1968), a progressivement envahi le littoral du nord-ouest africain, du Ghana en 1972 au Sénégal en 1978, pour atteindre le sud de la Mauritanie à partir de 1979, au point de constituer la moitié de la biomasse en poisson du plateau continental. Cette apparition peut être liée à un important changement dans la composition des communautés de poisson infra et supra-thermocline dont se nourrit cette espèce, en relation avec d'amples variations hydroclimatiques. D'autres phénomènes d'importance se rapportent au déplacement progressif vers le sud de la limite de migration des stocks de sardines du Maroc (*Sardina pilchardus*) entre les années 1969 et 1976 (Domanevski et Barkova 1978), où à la profonde transformation du type de pêche pratiquée au large du Sénégal (Garcia *et al.* 1978) : la composition spécifique des débarquements démersaux à Dakar, basés essentiellement sur les daurades et les pageots (environ 70 % des prises totales), a connu un brusque changement à partir de 1966 (fig. 9). De 1967 à 1972, les pêches ont surtout porté sur les crevettes et les soles, alors que les espèces précédemment dominantes ne représentaient plus que 10 à 15 % des débarquements. Ce changement, fréquemment expliqué par l'adaptation de la flottille de pêche aux conditions nouvelles du marché, a pu aussi être, au moins en partie, lié à une adaptation de la flottille aux stocks disponibles sur les mêmes lieux de pêche. Ainsi, on observe à partir de 1973 un retour progressif vers la situation originelle, bien que les prises de mérou ou thioff (*Epinephelus aeneus*) n'aient pas retrouvé leur niveau précédent. D'autres anomalies biologiques, comme leurs éventuelles relations avec les fluctuations des caractéristiques du milieu, ont été discutées par Boely (1978) et Freon *et al.* (1978) à propos de la pêche industrielle dakaroise de *Sardina aurita*.

Ces variations des caractéristiques des stocks sont souvent reliées aux modifications des caractéristiques du milieu, mais les explications sont peu developpées. Dans le secteur du cap Blanc, les années 60 ont été marquées par un spectaculaire ralentissement de l'activité de l'upwelling. Les conséquences écologiques entrainées par ce phénomène durable et intense (à la période de diminution de l'intensité de l'upwelling a succédé une longue période où l'activité du phénomène est restée minimale) sont difficilement appréciables. Aux effets écologiques proprement dits (variations de la productivité planctonique du milieu et donc de son potentiel nutritif, fluctuations thermiques et donc modifications probables des comportements liés à la migration et à la reproduction, compétition entre stocks pélagiques pour l'espace et les disponibilités trophiques), qui affectent le recrutement et la disponibilité des stocks, ont pu s'ajouter les conséquences de la surpêche observée dans certaines régions de l'ouest africain (Binet 1982), et donc profondément déséquilibrer les communautés de poissons présentes dans le milieu.

Remerciements

Ce travail a été mené dans le cadre de la coopération franco-mauritanienne. Je tiens à remercier M^me^ M. C. Bonin pour l'iconographie et M. B. Coste pour sa lecture critique du manuscrit.

Références

ANONYME. 1959. Instructions nautiques, série C(IV). Afrique, Côte Ouest (1^er^ volume). Au nord du cap des Palmes, îles Açores, de Madère, Canaries et du cap Vert. Service hydrographique de la Marine. Paris. 338 p.

BARTON, E. D., A. HUYER ET R. L. SMITH. 1977. Temporal variation observed in the hydrographic regime near Cabo Corveiro, in the north west African upwelling region, February to April 1974. Deep-Sea Res. 24 : 7–23.

BARTON, E. D., R. D. PILLSBURY ET R. L. SMITH. 1975. A compendium of physical observations from Joint I, vertical sections of temperature, salinity and sigma-t from R/V Gilliss data and low-pass filtered measurements of winds and currents. Oregon State University, School of Oceanography, Ref. 75-17 : 60 p.

BERRIT, G. R. 1961. Contribution à la connaissance des variations saisonnières dans le golfe de Guinée — Observations de surface le long des lignes de navigation. Première partie / généralités. Cah. Océanogr. 13(10) : 715–727.

1962. Contribution à la connaissance des variations saisonnières dans le golfe de Guinée — Observations de surface le long des lignes de navigation. Deuxième partie : étude régionale. Cah. Océanogr. 14(10) : 719–729.

1973. Recherches hydroclimatiques dans les régions côtières de l'Atlantique oriental — État des connaissances et perspectives. Bull. Mus. Natl Hist. Nat. Paris. 3^e^ série, n^0^ 148(4) : 85–99.

BINET, D. 1982. Influence des variations climatiques sur la pêcherie des *Sardinella aurita* Ivoiro-Ghanéennes : relation sècheresse surpêche. Oceanol. acta (4) : 443–452.

BOELY, T. 1978. Biologie de deux espèces de Sardinelles (*Sardinella aurita* Valenciennes 1847 et *Sardinella maderensis* Lowe 1841) des côtes sénégalaises. Thèse doctorale, Univ. Paris VI, Paris. 219 p.

CAVERIVIERE, A. 1982. Le baliste des côtes africaines (*Balistes carolinensis*). Biologie, prolifération et possibilités d'exploitation. Oceanol. acta 5(4) : 3–459.

CODISPOTI, L. A., R. C. DUGDALE ET H. J. MINAS. 1982. A comparison of the nutrient regime off Northwest Africa, Peru and Baja California. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 180 : 184–201.

COSTE, B. ET H. J. MINAS. 1982. Analyse des facteurs régissant la distribution des sels nutritifs dans la zone de remontées d'eau des côtes mauritaniennes. Oceanol. Acta 5(3) : 315–324.

DOMANEVSKI, L. N. ET N. A. BARKOVA. 1978. Particularités de la répartition et état des stocks de sardines (*Sardina pilchardus*) au large de l'Afrique nord-occidentale, p. 86–91. *Dans* Rapport COPACE 78/10, groupe de travail *ad hoc* sur les poissons pélagiques côtiers ouest africains de la Mauritanie au Libéria (26° N et 5° N) PNUD-FAO.

FRAGA, F. 1974. Distribution des masses d'eau dans l'upwelling de Mauritanie. Tethys 6(1–2) : 5–10.

FREON, P., B. STEQUERT ET T. BOELY. 1978. Les pêches sénégalaises. Description et analyse des captures et des rendements des principales espèces pélagiques côtières, p. 27–62. *Dans* Rapport COPACE 78/10, groupe de travail *ad hoc* sur les poissons pélagiques côtiers ouest africains de la Mauritanie au Libéria (26°N et 5°N) PNUD-FAO.

FURNESTIN, J. 1959. Hydrologie du Maroc atlantique. Rev. Trav. Inst. Pêches Marit. 23 : 5–78.

GARCIA, S., F. LHOMME, J. CHABANE ET C. FRANQUEVILLE. 1978. La pêche démersale au Sénégal : historique et potentiel. Doc. FAO-CECAF 78/8 : 58–76.

HALPERN, D. 1977. Description of wind and of upper ocean current and temperature variations on the continental shelf off Northwest Africa during March and April 1974. J. Phys. Oceanogr. 7(3) : 422–430.

HIDY, G. M. 1972. A view of recent air–sea interaction research. Bull. Am. Meteorol. Soc. 53 : 1083–1102.

HUNTSMAN, S. A. ET R. T. BARBER. 1977. Primary production off north west Africa : the relationship to wind and nutrient conditions. Deep-Sea Res. 24 : 25–33.

JONES, P. G. W. 1972. The variability of oceanographic observations off the coast of NW Africa. Deep-Sea Res. 19 : 405–431.

JONES, B. H. ET D. HALPERN. 1981. Biological and physical aspects of a coastal upwelling event observed during March–April 1974 off Northwest Africa. Deep-Sea Res. 28 : 71–81.

LONGHURST, A. R. 1962. A review of oceanography of the Gulf of Guinea. Bull. Inst. Fr. Afr. Noire. T. XXIV, sér. A, 3 : 633–663.

MINAS, H. J., L. A. CODIPOSTI ET R. C. DUGDALE. 1982. Nutrients and primary production in the upwelling region off Northwest Africa. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 180 : 148–183.

MITTELSTAEDT, E. 1983. The upwelling area off northwest Africa — A description of phenomena related to coastal upwelling. Prog. Oceanogr. 12 : 307–331.

MITTELSTAEDT, E., R. D. PILLSBURY ET R. L. SMITH. 1975. Flow patterns in the north west African upwelling area. Result of measurements along 21–40° N during February–April 1974. Dtsch. Hydrogr. Z. 28 : 145–167.

PILLSBURY, R. D., J. S. BOTTERO, R. E. STILL ET E. MITTELSTAEDT. 1975. Wind, currents and temperature off Northwest Africa along 21°40′ N during Joint I, February–April 1974. Data Rep. Oreg. State Univ. : 62 p.

POSTEL, E. 1968. Hydrologie et biogéographie marines dans l'ouest africain. *Dans* West African International Atlas, OAU/IFAN, Dakar : 13-A.

SCHEMAINDA, R. ET D. NEHRING. 1975. The annual cycle of the space temporal dislocation of the Northwest African upwelling region. Comm. n° 42, 3rd Int. Symp. Upwelling Ecosystems, Kiel, 25–27 August 1975.

SCHEMAINDA, R., D. NEHRING ET S. SCHULTZ. 1975. Ozeanologische untersuchungen zum produktionpotential des nord-west africanischen Wasserautriebs region 1970–1973. Geod. Geophys. Veroff 4(16) : 85 p.

SEDYKH, K. A. 1979. Étude de l'upwelling près de la côte de l'Afrique du Nord ouest par l'Atlant-NIRO. FAO/COPACE, 78/11 : 93–99.

SMALL, L. F. ET D. M. MENZIES. 1981. Patterns of primary productivity and biomass in a coastal upwelling region. Deep-Sea Res. 28 : 123–149.

SPETH, P. ET A. KHONE. 1983. The relationship between sea surface temperatures and winds off Northwest Africa and Portugal. Oceanogr. Trop. 18(1) : 69–80.

SPETH, P., H. DETLEFSEN ET H. W. SIERT. 1978. Meteorological influence on upwelling off Northwest Africa. Dtsch. Hydrogr. Z. (A) Hamb. 31 : 95–104.

STUART, D. W., T. BUCHWALTER ET J. GARCIA-MEITIN. 1975. Joint I meteorological data, Florida State University Data Report CUEA 75-3.

SVERDRUP, H. V., M. V. JOHNSON ET R. H. FLEMING (ÉD.). 1942. The oceans. Prentice Hall, Englewood Cliffs, NJ. 1087 p.

TOMCZAK, M. JR. 1977. Continuous measurements of near-surface temperature and salinity in the NW African upwelling region between Canary Islands and Cap Vert during winter of 1971–1972. Deep-Sea Res. 24 : 1103–1119.

1978. De l'origine et la distribution de l'eau remontée à la surface au large de la côte nord-ouest africaine. Ann. Hydrogr. 6, Sér. 5 : 5–14.

WILLIAMS, F. 1968. Report on the Guinean Trawling Survey. Organization African Unity, Lagos (Nigeria). Sci. Tech. Res. Comm. Publ. 99, 3 vol. : 828, 529 et 541 p.

WOOSTER, W. S., A. BAKUN ET D. R. MCLAIN. 1976. The seasonal upwelling cycle along the eastern boundary of the North Atlantic. J. Mar. Res. 34(2) : 131–141.

Influence of Snowcover Development and Ground Freezing on Cation Loss from a Wetland Watershed during Spring Runoff

D. C. Pierson[1] and C. H. Taylor

Watershed Ecosystems Program, Trent University, P.O. Box 4800, Peterborough, Ont. K9J 7B8

Pierson, D. C., and C. H. Taylor. 1985. Influence of snowcover development and ground freezing on cation loss from a wetland watershed during spring runoff. Can. J. Fish. Aquat. Sci. 42: 1979–1985.

Cation and water export were monitored from a small wetland watershed in south-central Ontario during spring runoff for two years with very different sequences of snowpack development. In 1977, a persistent snow cover developed in the watershed by 1 December, and snow accumulation was near normal for this region. In 1980, persistent snow cover did not develop until mid-January, and the final snowpack was well below average. Between years, major differences in the timing of ion export were found for calcium, magnesium, and sodium, ions which appear to be associated with the watershed's soils. Conversely, potassium, a cation not as strongly associated with soil ion sources, showed temporal patterns of export which were similar in timing during the two years. We hypothesize that concrete soil frost, which developed in 1980 as a consequence of the late-developing snow cover, strongly influenced the timing of ion export by isolating early spring runoff from the watershed's soils. Streamwater hydrogen ion concentrations decreased rapidly during the first week of spring runoff in 1980, indicating that hydrogen ion export was increased by a similar mechanism. We suggest that in poorly buffered systems, the formation of concrete soil frost may lead to significant increases in hydrogen ion export during spring snowmelt.

Les déperditions de cations et d'eau d'un petit bassin versant humide du centre-sud de l'Ontario ont été contrôlées au cours du ruissellement de printemps de deux années présentant des enneigements très différents. En 1977, une couverture de neige persistante était déjà en place le 1er décembre et l'accumulation de neige correspondait presque à la normale pour cette région. En 1980, la couverture nivale persistante n'est apparue qu'à la mi-janvier et l'accumulation de neige annuelle a été bien au-dessous de la moyenne. On a noté entre ces années d'importants écarts quant au moment des déperditions en ions calcium, magnésium et sodium liés aux sols du bassin versant. Au contraire, l'allure temporelle des pertes de potassium, un cation non aussi fortement lié aux sources ioniques du sol, était semblable au cours des deux années. Nous croyons que le gel total du sol survenu en 1980 suite à l'apparition tardive de la couverture nivale a fortement influé sur le moment des pertes ioniques en isolant le ruissellement de printemps des sols du bassin. La concentration en ions hydrogène des eaux du cours d'eau a rapidement diminué au cours de la première semaine du ruissellement de printemps de 1980; ce qui indique que les pertes d'ions hydrogène étaient favorisées par un mécanisme semblable. Nous formulons l'hypothèse que, dans les systèmes mal tamponnés, le gel complet du sol peut être cause d'une augmentation appréciable des pertes d'ions hydrogène au cours de la fonte des neiges du printemps.

Received February 4, 1985
Accepted August 13, 1985
(J8099)

Reçu le 4 février 1985
Accepté le 13 août 1985

Ion export from watershed ecosystems is known to be temporally variable. This variability may (e.g. Lewis and Grant 1979), or may not (e.g. Schindler et al. 1976), be simply related to annual variations in a watershed's hydrological budget. In cold climates, variations in the timing and extent of snow cover development are important environmental factors which must play a role in causing annual fluctuations in ion export. Snow accumulation over the winter strongly affects the magnitude of ion export occurring the following spring by providing a source of water which rapidly passes through a watershed with the onset of spring melt, and in doing so, transports ions from the basin. Our objective was to examine the influence of the timing of snow cover development on soil weathering and ion exchange processes which regulate ion loss through their effects on stream water chemistry. We hypothesize that the timing of snow cover development relative to declining winter temperatures is an important factor regulating stream water chemistry. If an insulative snow cover does not develop until well after the onset of subfreezing temperatures, we propose that the formation of concrete soil frost reduces stream water ion concentrations during early spring streamflow, by isolating soil ion sources from snowmelt runoff.

Research on the relationship between variations in snow cover development and ion export is sparse. Likens et al. (1977) presented data which indicate that soil freezing may strongly increase nitrate loss. During years when persistent snow cover did not develop until late in the winter and frozen soils were widespread, large increases in streamwater nitrate concentrations were observed. These increased concentrations persisted long after the winter, and resulted in large annual increases in nitrate export. Likens et al. (1977) suggested that soil freezing

[1]Current address: Division of Hydrology, Uppsala University, Västra Ågatan-24, Uppsala 5-752 20, Sweden.

may have induced soil nitrification, thereby increasing soil nitrate loss; however, the persistence of the elevated concentrations could not be explained. Lewis and Grant (1980) investigated ion loss during the fall–winter period prior to spring melt, when stream discharge is low. They found that in a year with below average snow cover and above average soil frost development, the loss of biologically important ions was increased. While they could not describe an exact mechanism to explain the elevated ion loss, their data strongly suggest that the frozen soils interfered with the biological sequestration of nutrient ions. The research presented here, while similar in scope to that of Likens et al. (1977) and Lewis and Grant (1980), differs in several important aspects. First, our work was carried out in a watershed of radically different topography, geology, and hydrology where soil frost might influence ion export in a different manner. Second, we only concerned ourselves with the influence of snow cover development on ion export occurring during the spring runoff period.

Study Basin and Methods

We compared hydrologic and ion export data collected during the spring of two years with markedly different winter seasons and patterns of snowpack development. This was done using the data of Wolfe (1980) and Pierson (1983), collected from a small (47.5 ha) wetland watershed which is located 5.6 km northwest of Peterborough, Ontario (Fig. 1).

The Telford watershed is in rural land use with 50% of its area being largely forested with white cedar (*Thuja occidentalis*) and sugar maple (*Acer saccharum*). Some oak (*Quercus* spp.), elm (*Ulumus* spp.), and ash (*Fraxinus* spp.) are also present. Of the remaining land, 35% is in pasture and 15% is in cultivated fields. The watershed is characterized by low relief and readily saturated soils, and it is typical of small headwater drainage basins found in this region. A wetland occupies approximately 24% of the watershed's area (Fig. 1), and is a major source of streamflow.

The watershed's hydrology has been described in detail by Taylor (1982) and Taylor and Pierson (1985). Generally, most storm runoff is generated by direct precipitation (rainfall and snowmelt) onto the wetland and areas of saturated soil adjacent to the stream. This precipitation, being unable to infiltrate into the soil profile, moves overland to the stream channel by the runoff pathway known as saturation overland flow (Dunne et al. 1975). Groundwater and subsurface flow are of less importance in generating storm flow, but do play an important role in sustaining discharge between storm events, especially in the area located downstream of the wetland. The runoff response of the watershed is therefore controlled largely by the areal extent of soil saturation which varies temporally in relation to variations in precipitation and evapotranspiration. As a result, the watershed has an ephemeral flow regime in which the spring is the major period of hydrologic export. Increasing levels of evapotranspiration reduce runoff as the spring proceeds, until runoff ceases in the late spring. During some years, when conditions allow for sufficient recharge of the swamp, stream discharge will occur in response to rainstorms in October and November. Runoff has never continued throughout the entire winter.

The bedrock of the area is Ordovician limestone overlain by Pleistocene glacial till, which varies in depth from 2 m in the lower part of the catchment to 30 m in the drumlin, which forms the southern drainage divide. The greatest relief in the basin is provided by this drumlin, which has an average slope of 8°. Otherwise, slopes average less than 3°. Within the wetland soils are highly organic mucks and peats which have cation exchange capacities of approximately 100 meq·100 g soil^{-1}. Outside the wetland, the soils are shallow loams or sandy loams which have cation exchange capacities of approximately 20 meq·100 g soil^{-1}. Soil base saturation typically exceeds 90% as a result of the calcareous composition of the bedrock and till.

Stream discharge was continuously monitored from the outlet of the watershed (Fig. 1) by use of a 90° V-notch weir equipped with a Belfort water level recorder. Water samples were collected from the weir at 1- to 7-d intervals depending on storm frequency and magnitude. The samples were analyzed for calcium and magnesium by atomic absorption spectrophotometry and for sodium and potassium by flame emission spectrophotometry (American Public Health Association 1975; Varian 1978).

To suppress anionic interferences in the calcium and magnesium determinations, and to prevent the precipitation of carbonate species, we added an acidic solution of lanthanum chloride to all sample aliquots. In 1980, sample pH was also determined using a Corning model 12 pH meter which was calibrated with standard buffer solutions. All pH measurements were made after a 10-min equilibration period in quiescent solutions. Usually, pH analyses were performed within 4 h and always within 24 h of sample collection.

In the Telford basin, stream concentrations varied independently of discharge under the conditions which accounted for the majority of water export. This necessitated calculating ion export over discrete time intervals determined by the frequency of sample collection. Ion export was therefore calculated as the product of the volume of water leaving the watershed between two consecutive sampling dates and the mean concentration of the two water samples. The resultant value was then expressed as an export rate (milliequivalents per hectare per hour), and these rates were summed over the entire spring runoff period to yield an overall export rate (equivalents per hectare per day). In a comparison of several commonly used methods for calculating watershed ion budgets, Scheider et al. (1979) found this method to yield highly accurate results.

Rainfall was measured with a siphoning recording rain gauge (Fig. 1) which recorded inputs that closely corresponded to those from a network of manual gauges.

Results and Discussion

The data in Fig. 2, collected at Trent University (6.8 km from the Telford watershed), show that the evolution of the final snowpack was extremely different for 1977 and 1980. Fourteen years of data (1970–83) collected from a 12-point snow course located at Trent University indicate a mean snow accumulation water equivalent of 144 mm. Snow accumulation was near normal in 1977 (138.5 mm) but much below average in 1980 (43.8 mm). Furthermore, persistent snow cover did not develop during the winter of 1980 until mid-January, which allowed ample opportunity for the soils to freeze. There were also greater amounts of rain in the fall and early winter of 1980 (Fig. 2), which led to areally extensive soil saturation. When saturated soils such as these freeze, a layer of concrete soil frost forms which is relatively impermeable to water infiltration. Excavations made in the pasture portion of the watershed in late January of 1980 showed concrete soil frost to extend 8–10 cm below the surface. In the winter of 1977, persistent snow cover

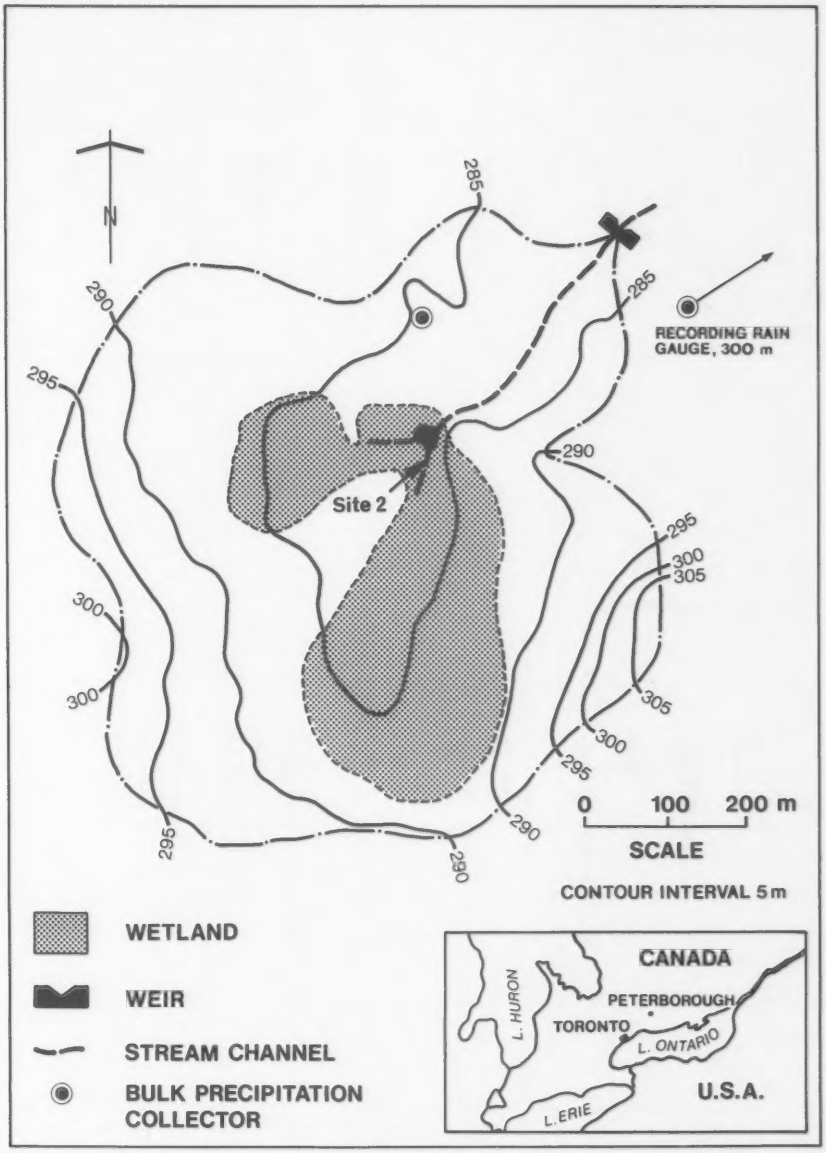


FIG. 1. Location of instrumentation and sampling sites within the Telford basin. Shaded area shows the extent of the wetland (standing water surrounded by saturated soil). Approximately 80% of the wetland is drained by the channel at site 2.

developed by 1 December. The insulative properties of this snow should have prevented, or greatly limited, the formation of soil frost during this winter. Adams and Barr (1976) and Adams (1978) have shown that, in the Peterborough area, surface soil temperatures remain at or only slightly below the freezing point throughout winters with sequences of snowpack development similar to 1977.

Stream hydrographs followed the same general pattern in both years (Fig. 3), although runoff began and ended somewhat earlier in 1977. Peak discharges were measured soon after the onset of runoff, as a result of snowmelt and rain events; then, as the spring progressed the runoff response to storm events decreased as the areal extent of soil saturation was reduced. Water budgets (precipitation input – stream output) calculated for both years were similar; in 1977, 49% of the precipitation input left the watershed via the stream, while in 1980, 46% did so.

Overall rates of cation export are presented in Table 1 which shows that calcium, magnesium, and potassium were exported at similar rates in 1977 and 1980, while in 1977, sodium was exported from the basin at approximately twice the rate as in 1980. Figure 3 presents the ion export data in more detail and

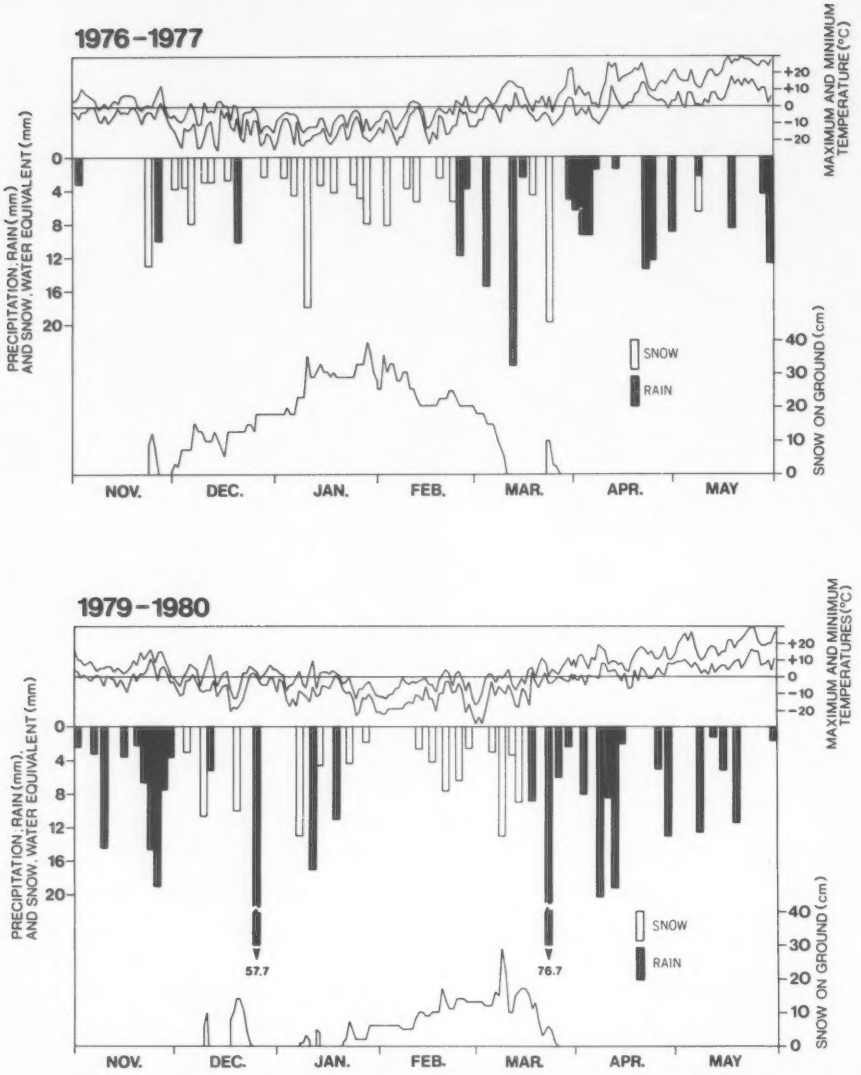


FIG. 2. Meteorological comparisons for the winter and spring of 1976–77 and 1979–80. Although the temperature regimes were similar in both winters, the snowpack developed much later and was shallower in 1980. Also, in 1979–80, 168.5 mm of rain fell prior to the development of persistent snow cover, while in 1976–77, only 13.4 mm of rain fell before a persistent snow cover had developed.

allows the importance of variations in stream chemistry in influencing ion export to be assessed. These data show that reduced concentrations of calcium, sodium, and, to a lesser extent, magnesium early in the runoff period of 1980 caused the majority of ion export to occur during a storm event in mid-April rather than during the initial peak discharges, as was the case in 1977. Potassium concentrations followed a similar pattern in 1977 and 1980. Early in the runoff period, concentrations were high, but they rapidly declined after 5–10 d of flow. This resulted in peak potassium export coinciding with peak discharge in both years.

The late development of a persistent snow cover in 1980 is the factor we feel to be responsible for the low concentrations and ion export rates of calcium, sodium, and magnesium measured during the early part of the runoff period that year. Dunne and Black (1971) have shown that concrete soil frost reduces soil infiltration capacity to very low levels. The lack of an insulative snow cover over much of the winter of 1980, and the resultant development of frozen soils, probably prevented early runoff water from infiltrating into the soil profile or displacing soil water into the stream. While we cannot describe the exact mechanism by which soil freezing reduced streamwater concentrations, isolation of ion sources located within the soil system in 1980 provides the most plausible explanation for the large

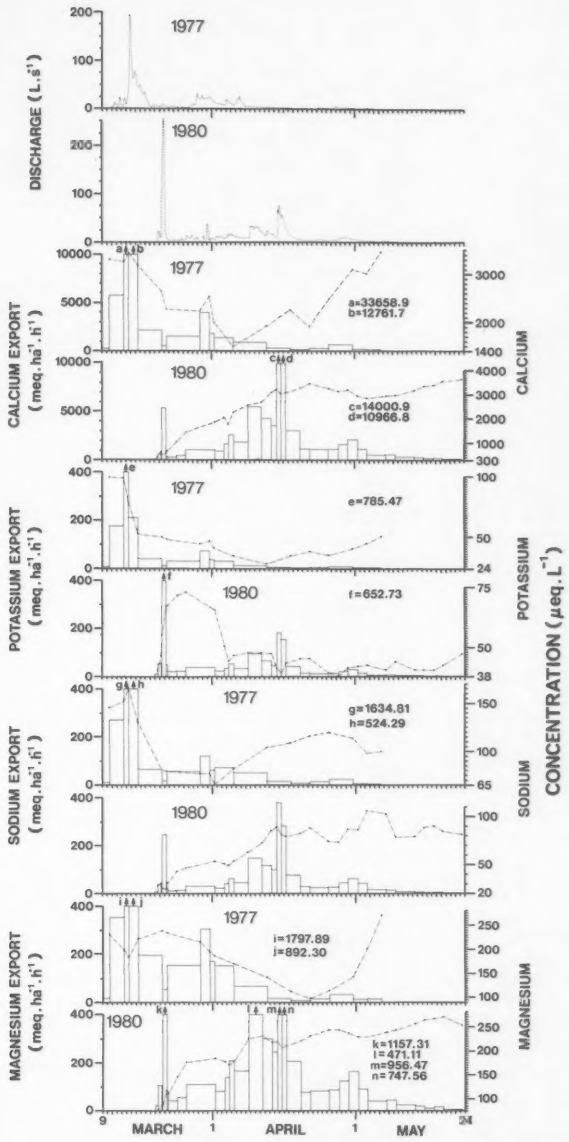


FIG. 3. Comparison of discharge, cation concentration, and cation export rates during spring runoff in 1977 and 1980. Concentrations are shown as a broken line. Export rates are shown as bars. Note the large differences in calcium, sodium, and magnesium concentrations and export rates measured during peak runoff in 1977 and 1980.

difference in the temporal pattern of stream chemistry between years.

The calcium and sodium concentrations of the samples collected during peak runoff in 1980 were only 14.4 and 15.7% of the concentrations of samples collected at peak runoff in 1977. As a result, ion export rates measured at peak discharge were 5.1 and 5.5 times greater in 1977 than in 1980 for calcium and sodium, respectively.

Chemical weathering of the calcareous bedrock and till underlying the basin provides a large supply of mobile calcium ions to the basin's soils and interstitial soil water. Typically, at least 90% of the soil's cation exchange capacities could be accounted for by exchangeable calcium (Pierson 1983). Isolation of the soil system from early snowmelt runoff would, therefore, almost certainly lead to lower streamwater calcium concentrations.

It is less obvious why sodium concentrations should be affected by soil frost development (Fig. 3), or why total sodium export showed the greatest contrast between years (Table 1). Atmospheric deposition can account for a large portion of the sodium export from the basin, with the net export probably being the result of losses arising from chemical weathering, organic deposition, or the export of mobile sodium ions retained within the basin between successive periods of stream flow. Exchangeable cation analysis has shown sodium to be only weakly associated with soil cation exchange sites. Mobile sodium ions are, however, present in the soils as soluble salts, and can be detected after extraction with distilled water. This suggests that the behaviour of sodium may at least partially be explained by the effect of frozen soils on the transport of interstitial soil water from the basin.

Potassium does not appear to be affected by the late-developing snowpack and frozen soils, as were the other cations. In both 1977 and 1980, streamwater potassium concentrations were highest during the early runoff period, which resulted in peak potassium export coinciding with peak stream discharge during both years. Potassium is strongly associated with organic sources, as it is readily leached from organic material (e.g. Attiwill 1968; Gosz et al. 1973). In the wetland, inputs of deciduous leaf litter to areas of standing water would be expected to provide potassium-rich water (Hodkinson 1975) which would be flushed from the watershed during early spring runoff. This leads us to believe that, even though potassium is present in the soils and soil water, standing water within the wetland is the major source of potassium to the stream during the first days of runoff. The trend in potassium concentration in 1980 is particularly indicative of potassium-rich water being flushed from the wetland. The first samples collected were low in potassium, since most runoff was generated by snowmelt in the open areas downstream of the wetland. Soon after the beginning of a large rain storm and increased melt, potassium concentrations increased rapidly as the wetland water was incorporated into the stream flow. Concentrations remained high for 10 d until the potassium-rich water was flushed from the basin, at which time the potassium concentrations declined sharply. Water samples collected within the wetland area from a flow channel which drained a large area of standing water (site 2, Fig. 1) further show the importance of the standing water as a source of potassium to the stream. During the first 10 d of runoff in 1980, samples from site 2 had a mean concentration of 86.3 $\mu eq \cdot L^{-1}$, which was significantly higher than 47.5 $\mu eq \cdot L^{-1}$ ($p = 0.005$), which was the calculated concentration of the water entering the stream downstream of site 2.

Magnesium appears to be both associated with soil sources and found at elevated concentrations in the standing water contained within the wetland. Although magnesium may be bound within cellular components of decomposing organic matter (Day and McGinty 1975), rapid losses of magnesium from decomposing litter have been measured by Gosz et al. (1973). It appears that magnesium enrichment of the standing water within the wetland by organic decomposition does occur, as concentrations at site 2 (mean concentration 164.4 $\mu eq \cdot L^{-1}$) were significantly greater ($p = 0.014$) than those calculated for

TABLE 1. Water and cation export rates from the Telford watershed measured over the spring runoff periods of 1977 and 1980.

	Duration of runoff (d)	Water (millions of $L \cdot ha^{-1} \cdot d^{-1}$)	Cation ($eq \cdot ha^{-1} \cdot d^{-1}$)				
			Calcium	Magnesium	Sodium	Potassium	Hydrogen
1977	59	0.019	52.7	3.70	2.26	1.10	NA
1980	65	0.018	41.0	3.44	1.14	0.90	0.154
Ratio 1980/1977		0.95	0.78	0.93	0.50	0.82	NA

the water entering the stream below the wetland (mean concentration 106.9 $\mu eq \cdot L^{-1}$). The average atomic ratio of Ca/Mg calculated from streamwater samples collected at the basin's outlet during the first 10 d of runoff ($n = 9$) was 6.79. In contrast, during the remainder of runoff ($n = 24$) the average streamwater Ca/Mg ratio was 13.17, which further suggests that the early runoff was enriched with magnesium from other than soil sources. The above data indicate that although magnesium concentrations during early runoff were reduced for similar reasons as applied to calcium and sodium, this effect is somewhat offset by inputs of magnesium-enriched water from the standing water in the wetland. Consequently, during runoff in 1980, magnesium concentrations increased from lowest to highest values by a factor of only 3.6, while calcium and sodium concentrations increased by factors of 9.7 and 6.6, respectively. This caused magnesium to show a response to soil freezing that was intermediate between that observed for calcium and sodium and that seen for potassium. As a result, the ion export peak for magnesium which occurred at the onset of runoff was of a similar magnitude to those obtained in mid-April (Fig. 3).

Because of this watershed's geology, hydrogen ion export is very low (Table 1) and stream water pH is usually neutral to slightly basic. However, hydrogen concentrations were highest in the first days of runoff (Fig. 4), and we expect that hydrogen ion export from the watershed was greater in 1980 for the same reason that calcium export was reduced, i.e. because water contact with highly buffered soils was limited by soil frost. This raises the possibility that in poorly buffered systems, the action of soil frost may be an important mechanism in regulating the delivery of hydrogen ions to aquatic ecosystems. In such a case, the isolation of already poorly buffered soils from waters moving over them may greatly reduce any potential of the terrestrial system to buffer acid meltwater pulses, thereby greatly increasing rates of acid loading.

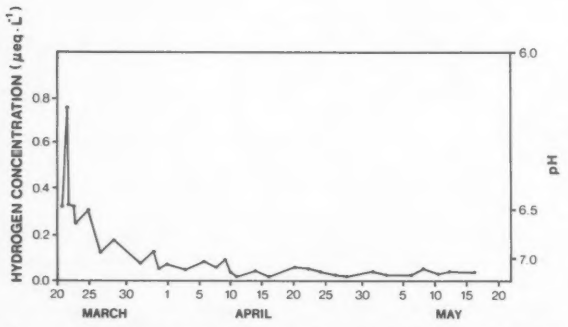


FIG. 4. Hydrogen concentrations measured at the basin's outlet during the spring of 1980. Even though the stream water was highly buffered, hydrogen concentrations were clearly elevated during the first 10 d of flow.

Our results indicate that a late-developing and below normal snowpack appreciably reduces streamwater concentrations of cations associated with soil or soil water sources, and as a result, cation export is greatly reduced during snowmelt runoff. Conversely, hydrogen ion concentration, which may be reduced through contact with exchangeable basic cations or carbonate compounds contained within the soil system, appears to be increased by the same phenomena, which should lead to increased hydrogen ion export during snowmelt runoff.

Acknowledgements

We wish to thank NSERC and Trent University for financial support, J. D. Wolfe for making his data available to us, and Mr. William Telford for providing access to his land. We also thank W. P. Adams and W. M. Lewis, Jr. for commenting on this manuscript.

References

ADAMS, W. P. 1978. The climatological record for Peterborough, Ontario. Occas. Pap. No. 6. Department of Geography, Trent University, Peterborough, Ont.

ADAMS, W. P., AND D. R. BARR. 1976. The evolution of snow cover in east-central Ontario with special reference to the international field year of the great lakes (IFYGL) and to stratigraphy. Proc. East. Snow. Conf. 33: 1–21.

AMERICAN PUBLIC HEALTH ASSOCIATION. 1975. Standard methods for the examination of water and waste water. 14th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC. 1193 p.

ATTIWILL, P. M. 1968. The loss of elements from decomposing litter. Ecology 49: 142–145.

DAY, F. P., AND D. T. MCGINTY. 1975. Mineral cycling strategies of two deciduous and two evergreen tree species on a southern Appalacian watershed, p. 736–743. *In* F. G. Howell, J. B. Gentry, and M. H. Smith [ed.] Mineral cycling in southeastern ecosystems. Rep. CONF-740513, National Techical Information Service, Springfield, VA.

DUNNE, T., AND R. D. BLACK. 1971. Runoff processes during smowmelt. Water Resour. Res. 7: 1160–1171.

DUNNE, T., T. R. MOORE, AND C. H. TAYLOR. 1975. Recognition and prediction of runoff producing zones in humid regions. Hydrol. Sci. Bull. 20: 305–327.

GOSZ, J. R., G. E. LIKENS, AND F. H. BORMANN. 1973. Nutrient release from decomposing leaf and branch litter in the Hubbard Brook Forest, New Hampshire. Ecol. Monogr. 43: 173–191.

HODKINSON, I. D. 1975. Dry weight losses and chemical changes in vascular plant litter of terrestrial origin, occurring in a beaver pond ecosystem. J. Ecol. 63: 131–142.

LEWIS, W. M. JR., AND M. C. GRANT. 1979. Relationships between stream discharge and yield of dissolved substances from a Colorado (USA) mountain watershed. Soil Sci. 128: 353–363.

1980. Relationships betweeen snow cover and winter losses of dissolved substances from a mountain watershed. Arct. Alp. Res. 12: 11–17.

LIKENS, G. E., F. H. BORMANN, R. S. PIERCE, J. S. EATON, AND N. M. JOHNSON. 1977. Biogeochemistry of a forested ecosystem. Springer-Verlag, Berlin, Germany. 146 p.

PIERSON, D. C. 1983. The role of hydrologic and chemical processes in determining cation export from a low relief wetland watershed. M.Sc. thesis, Trent University, Peterborough, Ont. 211 p.

SCHEIDER, W. A., J. J. MOSS, AND P. J. DILLON. 1979. Measurement and uses of hydraulic and nutrient budgets, p. 77–83. *In* Lake restoration: proceedings of a national conference. U.S. Environmental Protection Agency, Washington, DC.

SCHINDLER, D. W., R. W.NEWBURY, K. G. BEATY, AND P. CAMPBELL. 1976. Natural water and chemical budgets for a small Precambrian lake basin in central Canada. J. Fish. Res. Board Can. 33: 2526–2543.

TAYLOR, C. H. 1982. The effect on storm runoff response of seasonal variations in contributing zones in small watersheds. Nord. Hydrol. 13: 165–182.

TAYLOR, C. H. AND D. C. PIERSON. 1985. The effect of a small wetland on runoff response during spring snowmelt. Atmosphere-Ocean. (In press)

VARIAN. 1978. Analytical methods for flame spectroscopy. Publ. No. 85-100009-00. Varian Techtron Pty. Ltd., Springfield, Australia.

WOLFE, J. D. 1980. Chemical and hydrologic budgets of two small watersheds under different land use near Peterborough, Ontario. M.Sc. thesis, Trent University, Peterborough, Ont. 158 p.

Potential Usefulness of Chlorine for Controlling Pacific Salmon Leeches, *Piscicola salmositica*, in Hatcheries

S. M. Bower and L. Margolis

Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

and R. J. MacKay

Faculty of Mathematics, Department of Statistics and Actuarial Science, University of Waterloo, Waterloo, Ont. N2L 3G1

Bower, S. M., L. Margolis, and R. J. MacKay. 1985. Potential usefulness of chlorine for controlling Pacific salmon leeches, *Piscicola salmositica*, in hatcheries. Can. J. Fish. Aquat. Sci. 42: 1986–1993.

Susceptibility to the lethal effects of low levels of total residual chlorine (TRC) differed between juvenile coho salmon (*Oncorhynchus kisutch*) and the salmon leech (*Piscicola salmositica*), the vector for the haemoflagellate salmonid pathogen *Cryptobia salmositica*. There was no salmon mortality at concentrations below approximately 50 μg TRC/L for the maximum exposure time of 24 h. Some damage to gill tissue (hypertrophy, fusion, oedema, and some necrosis), was observed at the highest concentrations of TRC that did not kill any of the fish (approximately 50 μg/L for 24 h, and approximately 100 μg/L for 8 h). This damage was repaired by the fish within 4 d of exposure, although evidence of past irritation was noted in 6 of 12 fish. All small juvenile leeches exposed to 44 μg TRC/L for 24 h died, and over half of such leeches exposed to 44 μg/L for 8 h and 21 μg/L for 24 h died. Below TRC concentrations that were lethal to the fish (approximately 50 μg/L), contours of constant leech mortality derived from a logistic regression model fitted to the data offered a wide range of concentrations and exposure times that would result in high mortality of juvenile leeches. Larger subadult and adult leeches were more resistant to chlorine than the smaller leeches but were more sensitive than the juvenile fish. Thus, chlorine may prove useful for controlling *P. salmositica* in salmonid hatcheries.

La sensibilité aux effets létaux de faibles concentrations de chlore résiduel total (CRT) n'est pas identique chez le saumon coho juvénile (*Oncorhynchus kisutch*) et la sangsue du saumon (*Piscicola salmositica*), le vecteur de l'hémoflagellé *Cryptobia salmositica* pathogène pour les salmonidés. Les concentrations inférieures à environ 50 μg/L CRT n'ont pas provoqué de mortalité chez les saumons après une période d'exposition maximale de 24 h. On a cependant noté certaines lésions aux tissus des branchies (hypertrophie, fusion, oedème, et légère nécrose) aux concentrations les plus élevées de CRT ne provoquant aucune mortalité (environ 50 μg/L pendant 24 h ou 100 μg/L pendant 8 h). Les lésions étaient guéries moins de quatre jours après l'exposition, mais des indices d'une irritation antérieure pouvaient être notés chez 6 des 12 poissons. Toutes les petites sangsues juvéniles exposées pendant 24 h à une concentration de CRT de 44 μg/L sont mortes de même que plus de la moitié de celles exposées pendant 8 h à une concentration de 44 μg/L ou pendant 24 h à une concentration de 21 μg/L. Aux concentrations inférieures à la valeur létale pour les poissons (environ 50 μg/L) des courbes de valeurs constantes de mortalité obtenues par modèle de régression logistique ajusté aux données permettent d'obtenir une large gamme de concentrations et de temps d'exposition correspondant à une mortalité élevée des sangsues juvéniles. Les sangsues subadultes et adultes étaient plus résistantes au chlore que les plus petites, mais elles étaient quand même plus sensibles que les poissons juvéniles. Le chlore pourrait donc être utilisé pour lutter contre *P. salmositica* dans les installations de production salmonicoles.

Received October 16, 1984
Accepted August 16, 1985
(J7976)

Reçu le 16 octobre 1984
Accepté le 16 août 1985

The rhynchobdellid leech *Piscicola salmositica* Meyer is the only vector of the haemoflagellate *Cryptobia salmositica* Katz (Becker and Katz, 1965, 1966). This haemoflagellate has been identified as a lethal pathogen of salmonids in culture or seminatural rearing facilities on the Pacific coast of North America (Wales and Wolf 1955; Wood 1979; Mundie and Traber 1983; Bower and Margolis 1983). One possible method of eliminating this pathogen is to control the leech vector.

Piscicola salmositica itself can be a problem when present in large numbers. Losses as high as 25% were estimated for pink salmon fry (*Oncorhynchus gorbuscha*) in some troughs in a hatchery in Washington State due to the feeding activities of this leech (Earp and Schwab 1954). Wood (1979) also suggested that extremely heavy infections of *P. salmositica* can kill adult Pacific salmon by physically blocking the circulation of water over the gills or by direct injury to the gill tissue.

Earp and Schwab (1954) found that formalin, Roccal, and pyridylmercuric acetate, used in the 1950s to control bacterial and parasitic diseases of Pacific salmon, had no effect on leeches but an exposure of 1 h in undiluted seawater (salinity at least 28‰) killed all leeches. Since treatment with seawater is not feasible in most salmon hatcheries, other means of controlling this parasite are required. Development of a successful

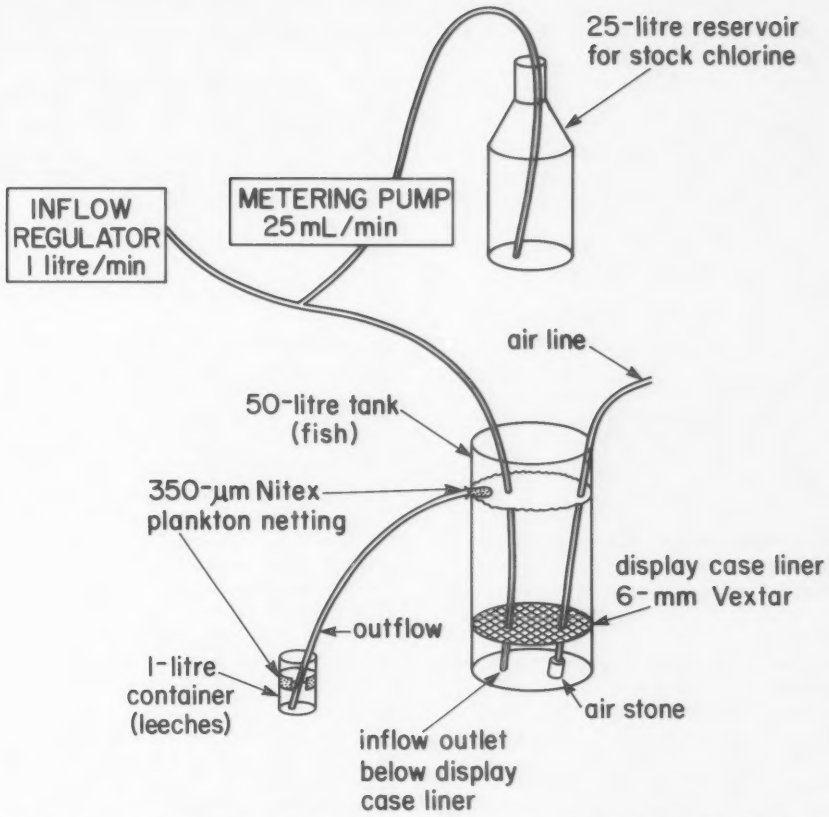


FIG. 1. Design of experimental tanks used to expose juvenile *Oncorhynchus kisutch* and *Piscicola salmositica* to sodium hypochlorite.

treatment for leeches would not only control the leech but also the haemoflagellate it transmits.

Beeton et al. (1976) reported that the abundance of leeches (as well as other invertebrates) was reduced in areas of Milwaukee Harbor and adjacent areas of Lake Michigan exposed to chlorine effluent from a sewage treatment plant, and they suggested that chlorine toxicity may account for this reduction. During recent studies on the biology of *P. salmositica* it was noted by one of us (S.M.B.) that newly hatched leeches maintained in a dechlorinated water supply at the Pacific Biological Station in Nanaimo, British Columbia, did not survive whereas juvenile salmon in the same water thrived. Records of total residual chlorine (TRC) concentration of the dechlorinated water indicated that on 14 d between August 17 and October 21, 1983, TRC levels rose to between 10 and 40 µg/L for periods lasting from 2 to 24 h (John Blackburn, Pacific Biological Station, pers. comm.). During the same period, *P. salmositica* were cultured successfully in an unchlorinated groundwater supply at the Rosewall Experimental Hatchery on Vancouver Island, British Columbia.

Our experiments were designed to compare the sensitivity of *P. salmositica* and juvenile coho salmon (*Oncorhynchus kisutch*) to low chlorine levels and to determine if chlorine could be used for controlling leeches in Pacific salmon hatcheries.

Materials and Methods

Three chlorine exposure experiments were conducted in groundwater at the Rosewall Experimental Hatchery. Sixteen 50-L flow-through fiberglass tanks were used in each experiment. Individual tanks (Fig. 1) received 1 L of water per minute. The water inflow pipe and air supply were delivered to the bottom of each tank and held in place by a stiff plastic (Vexar) display case liner (6-mm mesh). The water outflow, located at the top of each tank, was covered with plankton netting (350-µm Nitex).

Chlorine (sodium hypochlorite obtained as a 10.8% solution, Guardsman-12, Van Waters and Rogers Ltd., Vancouver, B.C.) was mixed in water to the desired concentration and placed in 25-L small "necked" reservoirs and introduced into the tanks via a Y junction on the inflow tube. The diluted chlorine was metered into the inflow tube at approximately 25 mL/min by using metering pumps (Flow Inducer model MHRE/200, manufactured by Watson-Marlow Ltd., Cornwall, England). TRC concentrations in the fish tanks were monitored frequently throughout the chlorine exposure period using a Chlortect Chlorine Monitor (model No. 3500, EPCO Ind., Woodbury, CT) calibrated to measure 1 µg chlorine/L (±2%) (Sellner and Marinenko 1981). In most tanks, TRC readings were taken

every 1–1.5 h for the first 10 h of exposure and again within the last 30 min before the chlorine flow was turned off. If the TRC concentrations were above or below the desired level, the metering pumps were adjusted to correct the flow from the chlorine reservoir tank and readings were taken every 15–20 min until the desired level was reached. The average TRC concentration in each tank was determined by graphing all readings taken from each tank against time, calculating the area under the graph from the 1 h point to the time at which the chlorine flow was turned off, and dividing the area by the time period:

$$A = \frac{(c_1 + c_2)(t_2 - t_1)}{2} + \frac{(c_2 + c_3)(t_3 - t_2)}{2} + \ldots + \frac{(c_{i-1} + c_i)(t_i - t_{i-1})}{2}$$

where A is the area under the curve and $c_1, c_2, \ldots, c_i$ are the individual readings of TRC concentrations at times $t_1, t_2, \ldots, t_i$. The average TRC concentrations ($\bar{c}$) to which experimental animals were exposed, then, is

$$\bar{c} = A/t_j$$

where t_j is the number of hours during which chlorine was added minus 1 h for the initial adjustment period. These average TRC concentrations are used in reporting the results, and the range of TRC concentrations measured during the experiments appear in the appropriate tables.

Temperature and dissolved oxygen were measured when each experiment was in progress and four water samples were sent to EPS/DFO Laboratory Services (4195 Marine Dr., West Vancouver, B.C.) for organic component and metal analyses in order to describe water quality.

Each tank held between 14 and 26 juvenile coho salmon (9- to 15-cm fork length and 9- to 38-g wet weight). The fish were not fed while in the tanks. Each tank, and especially the plankton net over the outflow, was cleaned daily.

Three chlorine exposure experiments were conducted as described in the following paragraphs. Only a single exposure to chlorine was used in experiments 1 and 2 whereas fish and leeches were exposed to chlorine twice in experiment 3.

In experiment 1, approximately 20 small leeches (5–15 mm in length) were added to each of 16 tanks 1 d before introducing the fish. Eight days later, five groups of three tanks received target chlorine concentrations of 5, 10, 50, 100, and 500 μg/L, with each tank of a group exposed for 3, 8, or 24 h. One tank (control) received no chlorine. Following exposure, the chlorine was turned off and flushed from the flow-through system with the water exchange. Total fish mortalities were determined after 6 d of incubation. When the experiment was terminated on day 6, few leeches were recovered (13 of 320 placed in the tanks) and only two were found in the untreated tank. Because the leeches could not escape through the plankton netting, we believe they probably were eaten by the fish or possibly died and disintegrated. For experiments 2 and 3, leeches were placed in 1-L plastic containers clamped to the ends of the tank outflow lines (Fig. 1). Two transverse windows were cut in the sides of each container, such that the containers held approximately 0.5 L of water. Each window was covered by plankton netting (350-μm Nitex) glued to the outside of the container with black utility cement (B. F. Goodrich, Kitchener, Ont.) to contain the leeches.

In experiment 2, between 22 and 30 small leeches (5.2–19.0 mm in length, mean 12.1 ± 4.0 mm SD) were placed in each of the 14 plastic containers on the same day fish were placed in the tanks. Two days later, four groups of three tanks received target chlorine concentrations of 10, 25, 50, or 75 μg/L, with each tank of a group exposed for 3, 8, or 24 h. Two other tanks served as controls and received no chlorine. Dead fish and leeches were counted and measured daily. Observations were terminated 7 d after exposure to chlorine. All surviving leeches and fish were counted, subsamples were taken for size measurements, and the remaining animals were destroyed.

In experiment 3, between 34 and 45 subadult and adult leeches (13.7–30.5 mm in length, mean 24.0 ± 3.9 mm SD) were placed in each of 16 plastic containers on the same day that fish were introduced into the 16 tanks connected to the plastic containers. Two days later, chlorine was applied at target concentrations of 25 μg/L for 24 h or 50 μg/L for 8 h to seven tanks for each of the two concentration–time exposures, with two untreated tanks serving as controls. Seven days later, 12 tanks, 6 from each of the two levels of initial exposure, were treated with chlorine for a second time. Five of the tanks from each level of initial exposure were treated with one of the following target concentrations of chlorine: 100 μg/L for 8 h, 100 μg/L for 24 h, 75 μg/L for 8 h, 75 μg/L for 24 h, or 50 μg/L for 24 h. The other two tanks used in the initial exposures were exposed again at the same target chlorine concentration and time used during the initial exposure, i.e. 25 μg/L for 24 h and 50 μg/L for 8 h. During this second exposure, four tanks (one tank from each of the initial chlorine exposures and two tanks that were not originally treated) were retained as controls. Mortalities were recorded and the experiment terminated on day 7 as described earlier.

In experiments 1 and 2, immediately after chlorine exposure and again 4 d later, a total of 48 fish from 10 tanks were preserved in Bouin's fixative and the gills processed for histological examination. Sections were cut at 7 μm and stained with Harris' modified haematoxylin and 0.5% alcoholic eosin. Sections were examined with a light microscope at magnifications of 100–1000×. The fish collected for histological examination were not included in the final mortality counts and statistical analyses.

Results

Throughout all three experiments, the water temperature was 9 ± 1°C, oxygen concentrations varied between 10.2 and 10.9 mg/L, and the pH was between 6.8 and 6.9. The water was clear and based on four samples contained 3.2–3.6 mg sulfate/L, <0.005 mg nitrite/L, 0.14–0.15 mg nitrate/L, and 0.019–0.040 mg ammonia/L, with total organic carbon <1.0 mg/L and total inorganic carbon of 8.0 mg/L. Total water hardness ranged from 32.8 to 34.6 mg/L. Individual extractable metals ranging from highest to lowest concentrations were as follows: Ca, 10.1–10.6 mg/L; Si, 4.4–4.6 mg/L; Mg, 1.9 mg/L; Na, 1.3–1.7 mg/L; Zn, <0.002–0.17 mg/L; B, 0.02–0.07 mg/L; Sr, 0.012–0.013 mg/L; Cu, <0.005–0.007 mg/L; As, P, Sb, Se, Al, Ni, Pb, V, and Sn, <0.05 mg/L; and Co, Cr, Mo, Fe, Cd, Ti, Mn, Ba, and Be, <0.005 mg/L. When chlorine was added, each tank took about the same time to equilibrate to the desired chlorine concentration as it took to flush all the chlorine from the tanks once the chlorine was turned off. This time varied from about 25 min for 50 μg chlorine/L to about 60 min for concentrations of about 100 μg chlorine/L.

In all three experiments, results from fish exposures to similar average TRC concentrations were similar (Tables 1 and 2).

TABLE 1. Mortalities (no. of dead fish/no. of fish exposed) among juvenile *Oncorhynchus kisutch* 7 d after exposure to varying concentrations (μg/L) of sodium hypochlorite[a] (experiments 1 and 2; results of experiment 2 are in italics).

Period of exposure								
3 h			8 h			24 h		
Concentration[b]			Concentration[b]			Concentration[b]		
T	*A*	Mortalities	*T*	*A*	Mortalities	*T*	*A*	Mortalities
5	3 (0–4)	0/19	5	2 (1–4)	0/21	5	2 (1–6)	0/20
10	*3 (2–4)*	*0/20*	*10*	*5 (3–7)*	*0/20*	*10*	*5 (3–8)*	*0/16*
10	6 (5–7)	0/16	10	6 (4–8)	0/20	10	6 (4–7)	0/19
25	*19 (16–26)*	*0/20*	*25*	*21 (14–23)*	*0/20*	*25*	*21(17–26)*	*0/16*
50	*48 (44–54)*	*0/17*	*50*	*44 (39–48)*	*0/17*	*50*	*44(36–46)*	*0/17*
50	49 (30–61)	0/20	50	55 (40–63)	0/20	50	47(42–57)	0/19
75	*86 (64–94)*	*0/17*	*75*	*73 (47–100)*	*0/17*	*75*	*65(53–75)*	*10/17*
100	94 (64–105)	0/19	100	79 (64–88)	0/20	100	76(64–92)	21/21
500	525(405–611)	22/22	500	455(241–519)	49/49		—	—

[a]Of 60 fish in three control tanks not exposed to chlorine, none died.
[b]*T* = target concentrations; *A* = actual concentrations expressed as the average and range (in parentheses) of sodium hypochlorite concentrations measured during the exposure period.

TABLE 2. Mortalities (no. of dead fish/no. of fish exposed) among juvenile *Oncorhynchus kisutch* 7 d after an initial exposure and again 7 d after a second exposure to varying concentrations (μg/L) of sodium hypochlorite (experiment 3).

Initial exposure		Second exposure						
			8 h			24 h		
Concentration and time	Mortalities	None, mortalities	Concentrations[a]			Concentrations[a]		
			T	*A*	Mortalities	*T*	*A*	Mortalities
0	0/14	0/14	—	—	—	—	—	—
27(13–37) for 24 h	0/119	0/19				25	23 (19–24)	0/16
						50	51 (39–63)	0/14
			75	85 (74–104)	0/16	75	72 (65–87)	5/19
			100	114(102–128)	0/16	100	104 (94–123)	19/19
49(35–58) for 8 h	0/123	0/22	50	58 (49–63)	0/15	50	53 (46–65)	0/16
			75	78 (66–84)	0/16	75	70 (64–82)	1/16
			100	110(105–117)	0/16	100	108(104–111)	22/22

[a]*T* = target concentrations; *A* = actual concentrations expressed as the average and range (in parentheses) of sodium hypochlorite concentrations measured during the exposure period.

Although there was only one exposure to chlorine in experiments 1 and 2 (Table 1) and two exposures to chlorine 7 d apart in experiment 3 (Table 2), none of the fish exposed to 47 μg TRC/L or less for 24 h or up to 114 μg TRC/L for 8 h or less died. All fish exposed to more than 76 μg/L for 24 h or 525 μg/L for 3 h or longer died. Only some (26–59%) of the fish exposed to 65–72 μg chlorine/L for 24 h died. At these concentrations, fish with prior exposure to 49 and 27 μg chlorine/L for 8 and 24 h, respectively, 7 d earlier, had lower mortalities (6 and 26%, respectively) than fish with no prior exposure to chlorine (59–100% of the fish died).

No information was obtained on the susceptibility of leeches to chlorine in experiment 1 because of the exposure method used (see Materials and Methods). In experiment 2, death occurred in more than 50% of the small leeches exposed to 21 μg chlorine/L for at least 24 h, 44 μg chlorine/L for at least 8 h, and 86 μg chlorine/L for more than 3 h (Table 3). A logistic regression model was fitted to these data. In this model, the probability of death, p, of any individual is given as a function of the chlorine concentration c and the exposure time t, and is expressed as

$$p = \exp[f(c, t)]/(1 + \exp[f(c, t)])$$

where the function $f(c, t)$ takes the form

$$f(c, t) = \beta_0 + \beta_1 c + \beta_2 t + \beta_3 \log c + \beta_4 ct.$$

The computer package GLIM (Baker and Nelder 1978) was used to estimate the coefficients β_0, β_1, β_2, β_3, and β_4. All estimated coefficients were significantly different from 0 ($P < 0.05$, Table 4). The model fits the data well with the only real discrepancy being at $c = 25$, $t = 3$. To interpret these estimates, if time of exposure is held constant, mortality increases as concentration increases but, due to the negative coefficient for

TABLE 3. Mortalities (no. of dead leeches/no. of leeches exposed) among small *Piscicola salmositica* (5.2–19.0 mm in length) 7 d after exposure to varying concentrations (μg/L) of sodium hypochlorite[a] (experiment 2).

Period of exposure								
3 h			8 h			24 h		
Concentration[b]			Concentration[b]			Concentration[b]		
T	*A*	Mortalities	*T*	*A*	Mortalities	*T*	*A*	Mortalities
10	3 (2–4)	0/30	10	5 (3–7)	2/25	10	5 (3–8)	0/24
25	19(16–26)	3/22	25	21(14–23)	1/24	25	21(17–26)	13/25
50	48(44–54)	0/22	50	44(39–48)	19/28	50	44(36–46)	26/26
75	86(64–94)	21/28	75	73(47–100)	28/28	75	65(53–75)	28/28

[a]Of 51 small leeches in two control tanks not exposed to chlorine, two died.
[b]*T* = target concentrations; *A* = actual concentrations expressed as the average and range (in parentheses) of sodium hypochlorite concentrations measured during the exposure period.

TABLE 4. Estimated coefficients of the function $f(c, t) = \beta_0 + \beta_1 c + \beta_2 t + \beta_3 \log c + \beta_4 ct$ used in the model to describe the data in Table 3.

Coefficient	Effect	Estimate	SE
β_0	Intercept	5.250	3.686
β_1	Concentration	0.138	0.038
β_2	Time	−0.211	0.076
β_3	log *c*	−4.035	1.550
β_4	Total exposure	0.015	0.0035

log *c* (Table 4), at less than a linear rate. Conversely, if concentration is held constant, mortality increases as time of exposure increases, with the rate being greater for higher concentrations. From the model, values of *c* and *t*, for which the estimated mortality of leeches was constant, were calculated and contours of constant mortality were drawn for various probabilities (Fig. 2).

Sensitivity of large leeches (experiment 3) to TRC differed from that of small leeches. Two of the concentrations of chlorine (27 μg/L for 24 h and 49 μg/L for 8 h) that were lethal to at least 50% of the small leeches in experiment 2 killed less than 2% of the 558 subadult and adult leeches at both concentrations (Table 5). A second exposure to chlorine 7 d later resulted in high mortalities (>80%) at concentrations greater than 51 μg/L for 24 h and greater than 78 μg/L for 8 h. Since the data are sparse, a simple logistic model as above, which also included a third factor, initial exposure concentration *e*, was used such that

$$f(c, t, e) = \beta_0 + \beta_1 t + \beta_2 c + \beta_3 e.$$

There were no obvious departures from the model. All estimated coefficients were significantly different from 0 ($P < 0.05$) and the estimated coefficients for each factor were positive (Table 6), indicating increased mortality as the factor increased.

Gills from 6 of the 48 fish examined histologically were from tanks that did not receive sodium hypochlorite treatments (controls). These samples confirmed the healthy condition of the gills of fish used in these chlorine experiments. Gills from three fish preserved immediately after exposure to 21 μg chlorine/L for 24 h appeared normal. Of three fish examined histologically

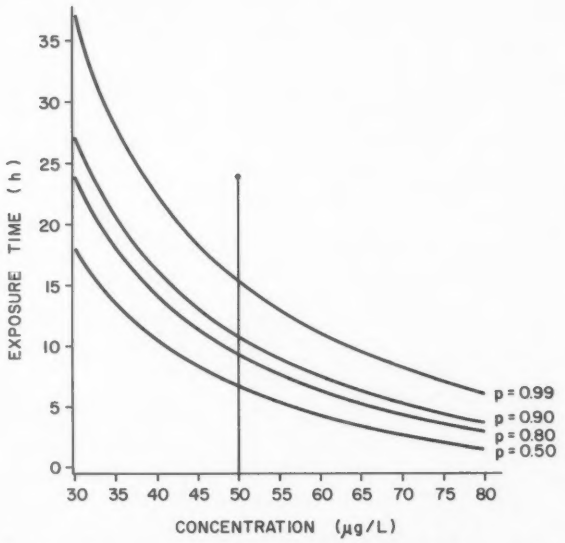


FIG. 2. Contours of constant mortality for exposed *Piscicola salmositica*. Any concentration and exposure time to the right and above the contours leads to an estimated mortality in small leeches (5–19 mm in length) greater than that associated with the contour. The line at 50 μg chlorine/L represents the chlorine concentration below which no fish died.

from the group exposed to 44 μg/L for 8 h, the gill tissue appeared normal in one, and showed patchy hypertrophy in two fish. At 55 μg/L the gills of three fish examined histologically were generally hypertrophied with some associated oedema. At concentrations of approximately 50 μg TRC/L for 24 h and higher in which none of the fish died, all 21 fish examined showed evidence of acute irritation manifested by hypertrophy, fusion, oedema, and some necrosis of gill tissue. By day 4 after exposure, most of the damage caused by the higher concentrations of chlorine was repaired, and although 6 of 12 fish examined had normal gills, the other 6 showed evidence of past irritation manifested by slight hypertrophy and oedema, and telangiectasis in one fish.

TABLE 5. Mortalities (no. of dead leeches/no. of leeches exposed) among subadult to adult *Piscicola salmositica* (13.7–30.5 mm in length) 7 d after initial exposure and again 7 d after a second exposure to sodium hypochlorite of varying concentrations (μg/L) (experiment 3).

Initial exposure		Second exposure					
			8 h			24 h	
			Concentrations[a]			Concentrations[a]	
Concentration and time	Mortalities	None, mortalities	*T*	*A*	Mortalities	*T*	*A*	Mortalities
0	0/79	0/79		—	—		—	—
27(13–37) for 24 h	3/272	1/39				25	23 (19–24)	1/39
						50	51 (39–63)	33/41
			75	85 (74–104)	32/34	75	72 (65–87)	40/40
			100	114(102–128)	40/40	100	104 (94–123)	36/36
49(35–58) for 8 h	4/286	0/40	50	58 (49–63)	4/39	50	53 (46–65)	45/45
			75	78 (66–84)	35/39	75	70 (64–82)	41/41
			100	110(105–117)	38/38	100	108(104–111)	40/40

[a] T = target concentrations; A = actual concentrations expressed as the average and range (in parentheses) of sodium hypochlorite concentrations measured during the exposure period.

TABLE 6. Estimated coefficients of the function $f(c, t, e) = \beta_0 + \beta_1 t + \beta_2 c + \beta_3 e$ used in the model to describe the data in Table 5.

Coefficient	Effect	Estimate	SE
β_0	Intercept	17.85	2.38
β_1	Concentration	0.214	0.024
β_2	Time	0.324	0.045
β_3	Initial exposure	0.047	0.022

Discussion

In his review, Brungs (1973) indicated that chlorine in water may be present as free available chlorine in the form of hypochlorous acid (HOCl) or hypochlorite ions (OCl^-) or both. Chlorine will also react with inorganic and organic nitrogen (ammonia, nitrites, nitrates, amino acids, and proteins) to yield chloramines or with many other organic components as well as being reduced by chemicals such as sulfide, iron, and manganese (White 1972). Thus, the chlorine demand of water varies with the chemical content of the water, and the relative amounts of free residual chlorine and chloramines would be determined by pH, ammonia content of the water, and the amount of chlorine added (Holland et al. 1960; Beeton et al. 1976; Brooks and Bartos 1984). The water used in the present study contained small amounts of nitrite, ammonia, total organic carbons, iron, and manganese, and slightly higher concentrations of sulfate and nitrate. Thus, the water had some demand for chlorine. Measurements of TRC in the experimental tanks were used to ascertain the levels of chlorine to which the fish and leeches were exposed.

The toxicity of chlorine to aquatic life depends on the relative amount of free residual chlorine (HOCl and OCl^-) and type of chloramines (mono-, di-, tri-) present (Mattice and Tsai 1983; Brooks and Bartos 1984). The relative proportions of the various chlorine compounds present in the total residual chlorine depends on pH and ammonia concentration. Monochloramine and OCl^- (in the absence of sufficient ammonia) are present in alkaline water (pH 9.0), and dichloramine and HOCl (in the absence of sufficient ammonia) are present in acidic conditions (pH 5.0) (White 1972). Thomas et al. (1979), Mattice and Tsai (1983), and Brooks and Bartos (1984) reported that each of these chlorine compounds varied up to sixfold in relative toxicities. Hypochlorous acid and dichloramine were significantly more toxic than monochloramine and OCl^-, and the concentrations of these compounds that showed the highest toxicity varied among fish species (Brooks and Bartos 1984). Also, vertebrates and invertebrates respond differently to chlorinated effluents (Turner and Chu 1983). In the present study, the relative proportions of the chlorine compounds were not measured. However, the low levels of ammonia, nitrites, and nitrates and the slightly acidic condition of the water (pH 6.8–6.9) suggest that the chloramines were probably a mixture of monochloramine and dichloramine with any free residual chlorine present as HOCl (extrapolated from graphs of White 1972). Because water chemistry was similar in the three experiments, the chlorine toxicity to juvenile coho salmon was comparable among experiments. These results are consistent with those of Holland et al. (1960), who found that three species of juvenile salmon (*Oncorhynchus tshawytscha*, *O. gorbuscha*, and *O. nerka*) could survive exposures of about 50 μg chlorine/L for periods as long as 23 d. Buckley et al. (1976) also found that TRC concentrations of 30 μg/L for 12 d were sublethal although adverse haematological effects were detected.

Below TRC concentrations that were lethal to juvenile coho salmon (approximately 50 μg/L), a wide range of chlorine concentrations and exposure times can be used that result in high mortalities among juvenile leeches (Fig. 2). Thus, there appear to be sufficient differences in the susceptibility to chlorine between juvenile coho salmon and juvenile *P. salmositica* to regard chlorine as potentially useful for controlling leeches in a coho salmon hatchery if residual chlorine levels are carefully controlled.

Even if residual chlorine levels are carefully controlled, environmental factors such as temperature and oxygen may have an impact on fish survival at TRC concentrations found to

be sublethal in the present study (about 50 μg/L). Dandy (1972) indicated that at least one toxic action of chlorine on fish results from damage caused to gill tissue. Cairns et al. (1975) hypothesized that since chlorine damages the gills of fish, elevated temperatures, which lower dissolved oxygen and increase the respiratory demand for oxygen, would increase the toxicity of chlorine to fish. The experimental results of Stober and Hanson (1974) with pink salmon and chinook salmon (*O. tshawytscha*) in seawater and of Seegert et al. (1977) with yellow perch (*Perca flavescens*) in freshwater support this hypothesis. Furthermore, gill damage caused by chlorine may predispose the fish to infection by pathogens to which they would otherwise be resistant.. In the present study slight damage to gill epithelium was observed in fish that were exposed to sublethal TRC concentrations of approximately 50 μg/L, but none of the fish that survived exposure to chlorine acquired detectable diseases. The effect of temperature and dissolved oxygen was not examined, since all experiments were conducted at water temperatures and dissolved oxygen concentrations likely to be found in southern British Columbia hatcheries during the fall when leeches are hatching.

The location of leeches in the environment may affect their exposure and thus susceptibility to chlorine. *Piscicola salmositica* is haematophagous and usually attaches in the fin axilla, on the fins, and under the operculum of large fish. When feeding on smaller fish, they attach to any site on the body surface. These leeches readily move from fish to fish and can also be found on the substratum where adult leeches lay their eggs. In experiment 1, an attempt was made to expose the fish and leeches to chlorine together, thus giving the leeches the opportunity of attaching to the fish and feeding during the exposure period. Unfortunately, insufficient leeches were recovered to determine the effect of chlorine on them under these conditions. Thus, leeches were placed in containers separate from the fish in experiments 2 and 3 and the question of whether leeches would be more or less susceptible to chlorine if they were attached to the fish could not be examined.

Our results show that older leeches are more resistant to chlorine toxicity than younger leeches. This problem could be circumvented by repeating the chlorine treatments about 1 wk apart throughout the season of leech abundance (mid-September to mid-February: Becker and Katz 1966), which would destroy the young leeches as they hatch. However, no data are available on effects of repeated chlorine treatment on salmon. As shown in our study, 1 wk would be sufficient time for healing of chlorine-induced gill damage in juvenile coho salmon. The apparent increased resistance of juvenile coho salmon to chlorine after recovery from a sublethal dose (Tables 1 and 2) has not been reported by other workers. However, DeGraeve and Ward (1977) found that fathead minnows and lake trout (specific names not given) with previous exposure (longer than 2 h) to chlorinated and chlorobrominated effluent, respectively, were capable of tolerating higher levels of TRC or TRBC for longer periods of time than fish that were not previously exposed to either halogen. Further work is necessary to determine the significance of this observation on juvenile coho salmon.

Before using low levels of chlorine in hatcheries to control leeches, the susceptibility of adult salmon to chlorine requires further investigation because the period of leech abundance also coincides with the return of adult salmon to the hatchery for spawning. Brungs (1973) hypothesized that larger fish may be more susceptible to the lethal effects of chlorine than smaller fish because larger fish have less gill surface area per unit body size than smaller fish and therefore gill damage may have a greater impact on larger fish. If gill damage should occur while treating adult salmon, they may not be capable of repairing the damaged gills because these fish are thought to be physiologically compromised when preparing to spawn. This would reduce the number of repeated treatments that adult salmon could tolerate.

Because the control of leeches can be achieved with low levels of chlorine, chlorine-treated water from a hatchery would not have to be dechlorinated before release into a river, especially if the river flow was sufficient to dilute the hatchery effluent several times. However, if dechlorination is necessary, Beeton et al. (1976) and Seegert and Brooks (1978) recommended the use of sodium sulphite, which is relatively nontoxic to aquatic organisms. Seegert and Brooks (1978) found an adequate margin of safety with a ratio of sodium sulphite to chlorine of 6:1 (by weight).

Acknowledgments

We thank the following persons for their contributions during this study: R. M. Humphreys and the staff at the Rosewall Experimental Hatchery for their cooperation and advice during the experiments; Dave Whitaker and Gary Meyer for assistance with setting up the experimental apparatus; John Blackburn for assistance in standardizing the Chlorotect Chlorine Monitor; and John Bagshaw for preparing and examining histological sections of gill tissues. Drs. D. F. Alderdice and J. B. Sprague provided valuable comments on earlier drafts.

References

BAKER, R. J., AND J. A. NELDER. 1978. The GLIM system release 3: generalized linear interactive modeling. Numerical Algorithms Group, Oxford. 160 p.

BECKER, C. D., AND M. KATZ. 1965. Tranmission of the hemoflagellate *Cryptobia salmositica* Katz, 1951 by a rhynchobdellid vector. J. Parasitol. 51: 95–99.

1966. Host relationships of *Cryptobia salmositica* (Protozoa: Mastigophora) in a western Washington hatchery stream. Trans. Am. Fish. Soc. 95: 196–202.

BEETON, A. M., P. K. KOVACIC, AND A. S. BROOKS. 1976. Effects of chlorine and sulfite reduction on Lake Michigan invertebrates. Rep. No. EPA600/3-76-036. U.S. Environmental Protection Agency, Duluth, MN. 133 p.

BOWER, S. M., AND L. MARGOLIS. 1983. Direct transmission of the haemoflagellate *Cryptobia salmositica* among Pacific salmon (*Oncorhynchus* spp.). Can. J. Zool. 61: 1242–1250.

BROOKS, A. S., AND J. M. BARTOS. 1984. Effects of free and combined chlorine and exposure duration on rainbow trout, channel catfish, and emerald shiners. Trans. Am. Fish. Soc. 113: 786–793.

BRUNGS, W. A. 1973. Effects of residual chlorine on aquatic life. J. Water Pollut. Control Fed. 45: 2180–2193.

BUCKLEY, J. A., C. M. WHITMORE, AND R. I. MATSUDA. 1976. Changes in blood chemistry and blood cell morphology in coho salmon (*Oncorhynchus kisutch*) following exposure to sublethal levels of total residual chlorine in municipal wastewater. J. Fish. Res. Board Can. 33: 776–782.

CAIRNS, J., A. G. HEATH, AND B. C. PARKER. 1975. Temperature influence on chemical toxicity to aquatic organisms. J. Water Pollut. Control Fed. 47: 267–280.

DANDY, J. W. T. 1972. Activity response to chlorine in the brook trout, *Salvelinue fontinalis* (Mitchill). Can. J. Zool. 50: 405–410.

DEGRAEVE, G. M., AND R. W. WARD. 1977. Acclimation of fathead minnows and lake trout to residual chlorine and bromine chloride. J. Water Pollut. Control Fed. 49: 2172–2178.

EARP, B. J., AND R. L. SCHWAB. 1954. An infestation of leeches on salmon fry and eggs. Prog. Fish-Cult. 16: 122–124.

HOLLAND, G. A., J. E. LASATER, E. D. NEWMANN, AND W. E. ELDRIDGE. 1960. Toxic effects of organic and inorganic pollutants on young salmon and trout. Wash. Dep. Fish. Res. Bull. 5: 198–215.

MATTICE, J. S., AND S. C. TSAI. 1983. Total residual chlorine as a regulatory tool, p. 901–912. *In* R. L. Jolley, W. A. Brungs, J. A. Cotruvo, R. B. Cumming, J. S. Mattice, and V. A. Jacobs [ed.] Water chlorination

environmental impact and health effects. Vol. 4, Book 2. Environment health and risk. Ann Arbor Science publishers, Ann Arbor, MI.

MUNDIE, J. H., AND R. E. TRABER. 1983. Carrying capacity of an enhanced side-channel for rearing salmonids. Can. J. Fish. Aquat. Sci. 40: 1320–1322.

SEEGERT, G. L., AND A. S. BROOKS. 1978. Dechlorination of water for fish culture: comparison of the activated carbon, sulfite reduction, and photochemical methods. J. Fish. Res. Board Can. 35: 88–92.

SEEGERT, G. L., A. S. BROOKS, AND D. L. LATIMER. 1977. The effect of a 30-minute exposure of selected Lake Michigan fishes and invertebrates to residual chlorine, p. 91–100. *In* L. D. Jensen [ed.] Biofouling control procedures. Marcel Dekker Inc., New York, NY.

SELLNER, H. R., AND G. MARINENKO. 1981. Monitoring chlorine at trace levels, p. 717–721. *In* R. L. Jolley, W. A. Brungs, J. A. Cotruvo, R. B. Cumming, J. S. Mattice, and V. A. Jacobs [ed.] Water chlorination environmental impact and health effects. Vol. 4, Book 1. Chemistry and water treatment. Ann Arbor Science Publishers, Ann Arbor, MI.

STOBER, Q. J., AND C. H. HANSON. 1974. Toxicity of chlorine and heat to pink (*Oncorhynchus gorbuscha*) and chinook salmon (*O. tshawytscha*). Trans. Am. Fish. Soc. 103: 569–576.

THOMAS, P., J. M. BARTOS, AND A. S. BROOKS. 1979. Comparison of the toxicities of monochloramine and dichloramine to rainbow trout, *Salmo gairdneri*, under various time conditions. *In* R. L. Jolley, W. A. Brungs, R. B. Cumming, and V. A. Jacobs [ed.] Water chlorination environmental impact and health effects. Vol. 3. Proc. Third Conference on Water Chlorination: Environmental Impact and Health Effects. Ann Arbor Science Publishers, Ann Arbor, MI.

TURNER, A., AND A. CHU. 1983. Chlorine toxicity as a function of environmental variables and species tolerance. *In* R. L. Jolley, W. A. Brungs, J. A. Cotruvo, R. B. Cumming, J. S. Mattice, and V. A. Jacobs [ed.] Water chlorination environmental impact and health effects. Vol. 4, Book 2. Environment health and risk. Ann Arbor Science Publishers, Ann Arbor, MI.

WALES, J. H., AND H. WOLF. 1955. Three protozoan parasites of trout in California. Calif. Fish Game 41: 183–187.

WHITE, B. C. 1972. Chapter 4, Chemistry of chlorination, p. 182–227. *In* Handbook of chlorination. Van Nostrand Reinhold Company, New York, NY.

WOOD, J. W. 1979. Diseases of Pacific salmon: their prevention and treatment. 3rd ed. State of Washington Department of Fisheries, Hatchery Division, Olympia, WA. 82 p.

Comparative Analysis of Ionoregulation in Rainbow Trout (*Salmo gairdneri*) of Different Sizes Following Rapid and Slow Salinity Adaptation

C. E. Johnston and J. C. Cheverie

Biology Department, University of Prince Edward Island, Charlottetown, P.E.I. C1A 4P3

Johnston, C. E., and J. C. Cheverie. 1985. Comparative analysis of ionoregulation in rainbow trout (*Salmo gairdneri*) of different sizes following rapid and slow salinity adaptation. Can. J. Fish. Aquat. Sci. 42: 1994–2003.

Plasma ionic values and gill (Na^+,K^+)-ATPase activity were compared for rainbow trout (*Salmo gairdneri*) of three different fork lengths (<11, 11–15, and >15 cm) following transference (1) from freshwater directly into seawater of 28–29‰ (DTT), (2) from freshwater directly into an estuarine environment at Fortune Bay where salinity fluctuated primarily between 17 and 29‰ (DTF), (3) from freshwater into a salinity that slowly increased to 28‰ in 22 d (SAT), and (4) from freshwater into freshwater (FC). Trout of all sizes displayed better growth, lower mortality, and better ionoregulatory capacity as judged by plasma Na^+, K^+, and Cl^- concentrations and (Na^+,K^+)-ATPase activity in sea cages in Fortune Bay (DTF). The poorest ionoregulatory capacity and growth was in the direct transfer from freshwater to salinity of 28–29‰ (DTT). Failure of small trout to adapt to constantly high salinity of 28–29‰ was due to a slower development of (Na^+,K^+)-ATPase activity, excessively high plasma Na^+ and Cl^- concentrations, hemoconcentration, and tissue dehydration. Except for trout severely stressed by high salinity, growth in seawater was more favorable than in freshwater. Prince Edward Island estuaries are very favorable as adaptation and rearing areas for small and large rainbow trout.

Les teneurs ioniques du plasma et l'activité de l'ATPase-(Na^+,K^+) au niveau des branchies ont été comparées chez des truites arc-en-ciel (*Salmo gairdneri*) de trois différentes longueurs à la fourche (<11 cm, 11–15 cm et >15 cm) suite à leur transfert (1) directement d'eau douce en eau de mer de salinité de 28–29 ‰, (2) directement d'eau douce en milieu estuarien (baie Fortune) où la salinité fluctue généralement entre 17 et 29 ‰, (3) d'eau douce en une eau dont la salinité a atteint 28 ‰ en 22 jours et (4) d'eau douce en eau douce. Les truites de toutes les tailles ont présenté une meilleure croissance, une plus faible mortalité et une plus grande capacité de régulation ionique si l'on en juge par les teneurs plasmatiques en Na^+, K^+ et Cl^- et l'activité de l'ATPase-(Na^+,K^+) en parcs marins situés dans la baie Fortune. Les plus faibles valeurs de capacité de régulation ionique et de croissance ont été notées chez les poissons directement transférés de l'eau douce à une eau de salinité de 28–29 ‰. L'incapacité des petites truites à s'adapter à une salinité constamment élevée de 28–29 ‰ avait pour cause un plus lent accroissement de l'activité de l'ATPase-(Na^+,K^+), des teneurs plasmatiques en Na^+ et Cl^- excessivement élevées, l'hémoconcentration et la déshydratation tissulaire. À l'exception des truites fortement stressées par la haute salinité, la croissance a été supérieure en eau de mer. Les estuaires de l'île du Prince-Édouard constituent des zones très favorables à l'adaptation et à l'élevage des truites arc-en-ciel de petites et de grandes tailles.

Received April 17, 1985
Accepted August 13, 1985
(J8205)

Reçu le 17 avril 1985
Accepté le 13 août 1985

The commercial culture of rainbow trout (*Salmo gairdneri*) in freshwater ponds and marine coastal zones of Prince Edward Island has grown steadily since 1974. With this growth has come a greater interest in knowing the most effective and least expensive method for adapting and culturing trout in estuarine and coastal conditions. Achieving seawater tolerance of rainbow trout at an early age can reduce production costs and improve the return on investment. Sedgwick (1970) and Landless (1976) demonstrated that young rainbow trout grow better and have a better conversion of food in salt water than in freshwater. Better growth in salt water has also been shown for other salmon species (Canagaratnam 1959).

The ability of rainbow trout to adapt to seawater is dependent on the development of branchial ionoregulatory mechanisms. Such regulatory mechanisms appear to reside in the chloride cells of the gill epithelium (Keys and Willmer 1932) and require the enzyme (Na^+,K^+)-ATPase located in the basolateral region of the cell for ion transport. When rainbow trout are transferred from freshwater to seawater they lose water and become dehydrated. Ionic differences cause Na^+ and Cl^- to enter the body through diffusion or seawater drinking. Elevated plasma Na^+ and Cl^- levels stimulate the ion extrusion mechanism and induce an increase in (Na^+,K^+)-ATPase activity. Increased (Na^+,K^+)-ATPase activity is accompanied by a lowering of the plasma Na^+ and Cl^- levels and adaptation to the saline conditions. Adaptation to seawater is related to body size, for small trout adapt less well than large trout (Houston 1961; Eddy and Bath 1979; Bath and Eddy 1979; Jackson 1981). In earlier

work (Johnston et al. 1983) we demonstrated that small rainbow trout (mean fork length of 11.9 cm) could adapt by slow acclimation procedures and grow in salinities of 28–29‰ and that failure to adapt was not due initially to low gill ATPase activity but rather to short-term tissue dehydration and ionic interference of cellular function. Our objective was to evaluate the relationship of trout size to ionoregulatory function following slow and rapid salinity increases in a commercial culture setting.

Materials and Methods

In early May, 22 000 yearling and 13 000 fingerling rainbow trout were transported by truck in oxygenated tanks from Shamrock Springs Hatchery in Erin, Ontario, to the seawater acclimation site at Tracadie Bay, Prince Edward Island. Both fingerling and yearling trout were reared from eggs produced by a domestic stock at Spring Valley Trout Farm in Ontario. The stock was reared for 15 generations in freshwater in Ontario but the original parents were sea-running steelhead stock from Kelona, British Columbia.

Trout trucked to the seawater acclimation site at Tracadie Bay on 6 May were transferred to six outdoor circular holding tanks (diameter 4.57 m; depth 71 cm) and held in running (flow rate ~132 L/min), aerated, degassed well water until they were segregated into the experimental groups.

We allocated 3537 trout to four experimental groups with each group having three size lots (<11, 11–15, and >15 cm) as follows:

Experimental group	Experimental protocol	Number of trout, mean (range) fork length		
		<11 cm	11–15 cm	>15 cm
SAT	On 6 May, trout were exposed to a slow increase in salinity from freshwater to 26–28‰ during a 22-d period at the Tracadie Bay acclimation site. On May 28, all trout were transferred to seawater pens at Fortune Bay	200 8.7 (6.0–10.9)	200 12.0 (11.0–14.5)	200 16.7 (15.5–19.5)
DTT	On 17 May, trout were transferred rapidly from freshwater to seawater of 26–28‰ salinity at the Tracadie Bay acclimation site. On 28 May, all trout were transferred to seawater pens at Fortune Bay	401 8.2 (6.3–10.9)	350 12.2 (11.0–14.7)	386 16.9 (15.5–22.3)
DTF	On 17 May, trout in this group were transferred rapidly from freshwater at the Tracadie Bay acclimation site to seawater pens at Fortune Bay where salinities fluctuated daily between 15 and 29‰	400 8.8 (6.0–10.9)	400 12.2 (11.2–14.4)	400 17.2 (15.0–20.0)
FC	Trout were cultured in freshwater at Tracadie Bay until 28 May and then transferred to a a freshwater tank at the University of Prince Edward Island	200 8.7 (6.0–10.9)	200 12.2 (11.2–14.8)	200 16.9 (15.0–20.0)

Trout in groups SAT and DTT were each held during the seawater adaptation period at the Tracadie Bay site in circular center-draining tanks (diameter 4.57 m; depth 0.9 m). DTF and FC trout were also held until transfer to sea pens in Fortune Bay and the University of Prince Edward Island (UPEI) in circular center-draining tanks (diameter 2.4 m; depth 1.2 m and diameter 1.3 m; depth 0.7 m, respectively). Freshwater was pumped from a nearby well and passed through a degassing chamber before flowing into the holding tanks. The chemical characteristics of the freshwater and seawater at the Tracadie Bay site and UPEI were determined (Table 1). Water temperatures and salinities of each experimental group were monitored daily (Fig. 1). All experimental groups at Tracadie received natural lighting and photoperiod conditions. Trout were fed by hand twice daily with a diet of moist pellets. The food formulation was as follows: raw fish, 40% (mostly flounder: 95% *Limanda ferruginea* and 5% *Pseudopleuronectes americanus* and cod frames *Gadus morhua*); fish meal, 22%; cod oil, 6%; vitamin premix, 0.5%; wheat middlings, 31.5%. The amount of food offered daily was approximately 4–5% wet body weight. Prior to seawater exposure, all trout were vaccinated against vibrio with VAB-2 vaccine (Wildlife Vaccines Ltd., Colorado) using the immersion method.

On 17 May, trout in the experimental group DTF at Tracadie Bay were transported by truck in freshwater tanks to the Fortune Bay site were they were transferred to a rectangular floating sea pen (length 3.66 m; width 3.05 m; depth 2.5 m). This pen was subdivided into three smaller pens for each size lot.

On 28 May, trout in experimental groups SAT and DDT were transferred to the Fortune Bay site and placed in three rectangular pens. All trout were offered a moist diet two or three times daily until satiation. The amount of food offered per day was between 4 and 5% of the fresh weight of the fish.

On 28 May, the FC group at Tracadie Bay was transferred to a holding tank at the University. The holding tank was circular (diameter 1.3 m; depth 0.70 m) with a center drain and a flow

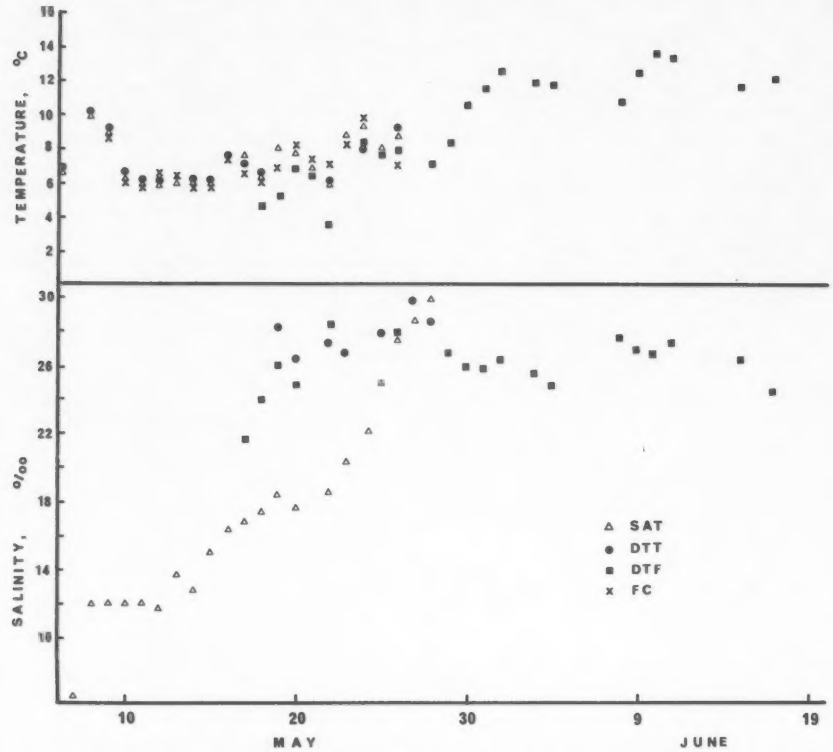


FIG. 1. Mean daily temperature and salinity conditions for each experimental regime: SAT, slow acclimation from freshwater to 28–29‰ salinity at Tracadie; DDT, direct transfer from freshwater to 28–29‰ salinity at Tracadie; DTF, direct transfer from freshwater to fluctuating salinity in Fortune River estuary; FC, freshwater control.

TABLE 1. Summary of the chemical characteristics of the freshwater and seawater used at Tracadie Bay and at the University of Prince Edward Island.

Chemical variable	Freshwater at Tracadie (mg/L)	Seawater at Tracadie (mg/L)	Freshwater at UPEI (mg/L)
NH_4-N[a]	<0.1	<0.1	<0.1
NO_3-N	0.4	<0.2	4.4
Cl	22	—	80.0
Ca	24	350.0	30.0
Mg	10.6	4200.0	18.4
P	<0.02	<0.01	0.16
Na	15.0	8200.0	31.0
K	2.2	390.0	3.4
Cu	0.05	—	<0.02
Pb	0.1	—	<0.01
Fe	<0.1	—	<0.01
Zn	<0.02	—	<0.02
Mn	<0.02	—	<0.02
SO_4	10.0	—	25.0
Total alkalinity (mg $CaCO_2$/L)	96.0	—	149.0
pH	7.8	8.2	7.2

[a]Water analysis was carried out by the Department of Agriculture and Forestry, Prince Edward Island Government.

of 12 L/min aerated freshwater. Trout were cultured under artificial fluorescent lighting and a natural photoperiod regime. Water temperatures were between 11 and 12°C and were similar to field conditions, but lacked daily fluctuations.

Initial measurements of body length and weight were made in early May and again at approximately 30-d intervals during and following the seawater adaptation period. Mortalities were recorded daily and the cumulative percent mortality was determined for each size lot and condition.

At different intervals during the seawater adaptation period, trout from each size lot were drawn at random and sampled for plasma Na^+, K^+, and Cl^- levels and branchial (Na^+,K^+)-ATPase activity. Because of hemoconcentration and a small plasma volume it was necessary to pool blood samples from several trout. Blood was collected and pooled from 2 trout in the large size lot, from 3–5 trout in the medium size lots, and from 10–15 trout in the small size lots. In each size lot a minimum of five pooled samples was used to determine the mean. Each trout sampled for blood was killed quickly by a blow to the head. After severing the caudal peduncle, blood was collected in lithium-heparinized blood collecting tubes. Collected blood was placed immediately in an ice bath. Blood plasma was separated from the blood cells by centrifugation at 2500 rpm for 3–5 min. Plasma was then withdrawn and used in ion determinations. Chloride determinations were made using a Buchler Chloridometer, while Na^+ and K^+ levels were determined using a Nova 1 Ion Analyzer.

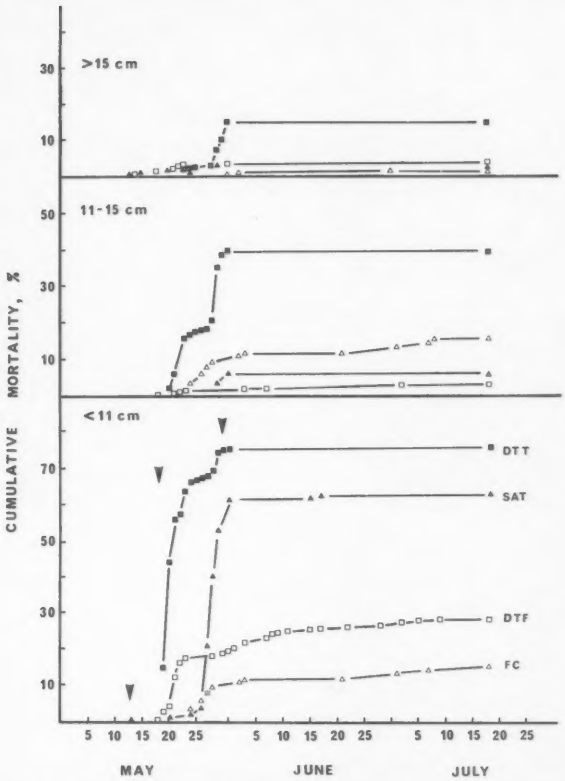


FIG. 2. Cumulative mortality of small (<11 cm), medium (11–15 cm), and large (>15 cm) rainbow trout before, during, and after seawater exposure in each experimental condition.

Trout sampled for blood plasma were used also for gill (Na^+,K^+)-ATPase assays. Five pooled samples were used in determining the mean ATPase level for each size lot. The number of fish used for each pooled gill sample varied from one to three, as more small trout were needed per sample than either large or medium trout. The methods used for ATPase determinations have been described by Johnston et al. (1983). ATPase activity was expressed as the micromoles phosphate (P_i) per milligram protein (P) per hour.

Statistical comparisons of ion and ATPase levels between size lots and adaptation conditions were carried out using analysis of variance and a Duncan's multiple range test or Student's *t*-test.

Results

Mortality

Mortality was greatest in all size lots of DTT regime (Fig. 2). Small trout were affected most by the high salinity and showed marked body dehydration, dispersed melanophores, elevated bile secretions, loss of feeding response, and a slow progressive loss of locomotor activity. Slightly more than 40% of the trout in the 11–15 cm group showed similar responses and died. Most of the dead trout were <13 cm in fork length. Seventeen percent of the trout >15 cm fork length also died within the first 7 d of exposure to high salinities.

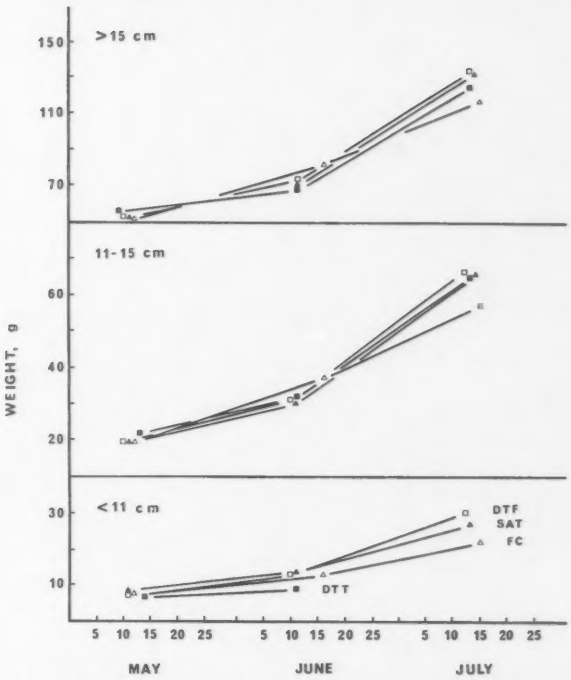


FIG. 3. Seasonal change in mean fresh weight of each size group in each experimental condition.

In the SAT regime there was a much lower mortality in all size groups than in the DTT regime. Small trout showed signs of salinity stress when salinities were above 18‰; mortality increased steadily as salinity rose to 26‰. Mortality levels for medium and large SAT trout were low and similar to freshwater and DTF groups.

Mortality levels for DTF trout were lower in small and medium size lots than in any of the other groups exposed to salt water. Tidal movements in the estuary created salinity changes that varied in depth and tidal cycle. On most days, salinity levels varied between 17 and 29‰, being greater near the bottom than at the surface. On several occasions, salinity levels at the surface dropped to 13‰ following a freshet. Although salinity levels were generally above 18‰ and as high as 29‰, small trout were not as stressed as those in SAT or DTT regimes.

Within the FC group there was mortality in the medium and small size groups. The cause of this mortality was not clearly evident. In both size groups most of the mortality occurred at the same time, approximately 20–30 d after trucking from Ontario. This mortality may represent a delayed response to shipping.

Growth

Growth was poorest during seawater adaptation in all size groups of the DTT regime (Fig. 3). However, following transfer to Fortune Bay on 28 May, growth improved and was better than trout in the FC regime. Medium and large trout in the FC regime initially showed better growth than any of the other groups exposed to seawater; however, by mid-July, trout in the FC regime were smaller and weighed less than those in seawater. In all size groups, growth in length and weight was greatest in the DTF group.

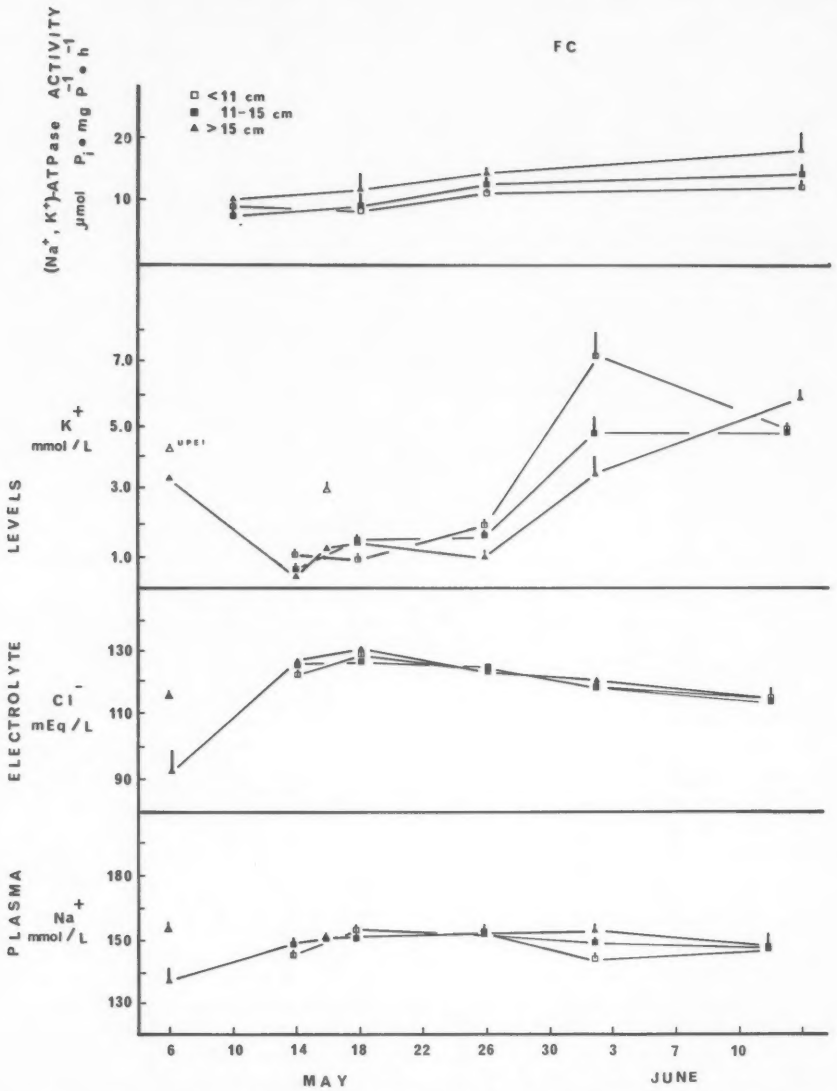


FIG. 4. Mean gill (Na^+,K^+)-ATPase activity and plasma K^+, Cl^-, and Na^+ concentrations (+0.5 SE) for each size group in the freshwater control conditions (FC). For comparison, mean plasma K^+, Cl^-, and Na^+ levels are shown for unstressed rainbow trout held at the University of Prince Edward Island.

Plasma Ion Concentrations and ATPase Activity

When trout arrived from Ontario at the acclimation site in Tracadie Bay, large trout in several of the carrier tanks showed signs of being severely stressed and many were moribund. Trout that had died before reaching Tracadie did not have flared opercular covers typical of anoxic conditions; therefore, a lack of oxygen during transit was not considered the cause of death. In an attempt to determine if shipping stress was the cause of death, blood samples were taken from 10 trout >15 cm fork length and assayed for ion concentrations. The mean plasma Na^+, K^+, and Cl^- levels were determined and are shown in Fig. 4. For comparison, plasma samples from three unstressed rainbow trout held in freshwater at UPEI were also assayed. Plasma ion concentrations in stressed rainbow trout were significantly lower ($p < 0.05$) than in unstressed rainbow trout at UPEI. Chloride levels were particularly low, being just above 90 mmol/L. Transfer of trout to freshwater holding tanks at Tracadie elevated both Na^+ and Cl^- concentrations to more normal values. However, K^+ levels declined from 3.3 to 0.5 mmol/L. Potassium levels remained low in all size groups cultured in freshwater at Tracadie. Blood samples taken from large trout at UPEI on 15 May had a K^+ level significantly higher ($p < 0.05$) than any of the trout groups at Tracadie. An analysis of the chemical composition of freshwater at Tracadie and UPEI showed a marked difference in ionic composition (Table 1). Freshwater at UPEI had higher concentrations of Na^+ and K^+ than freshwater at Tracadie. When rainbow trout were

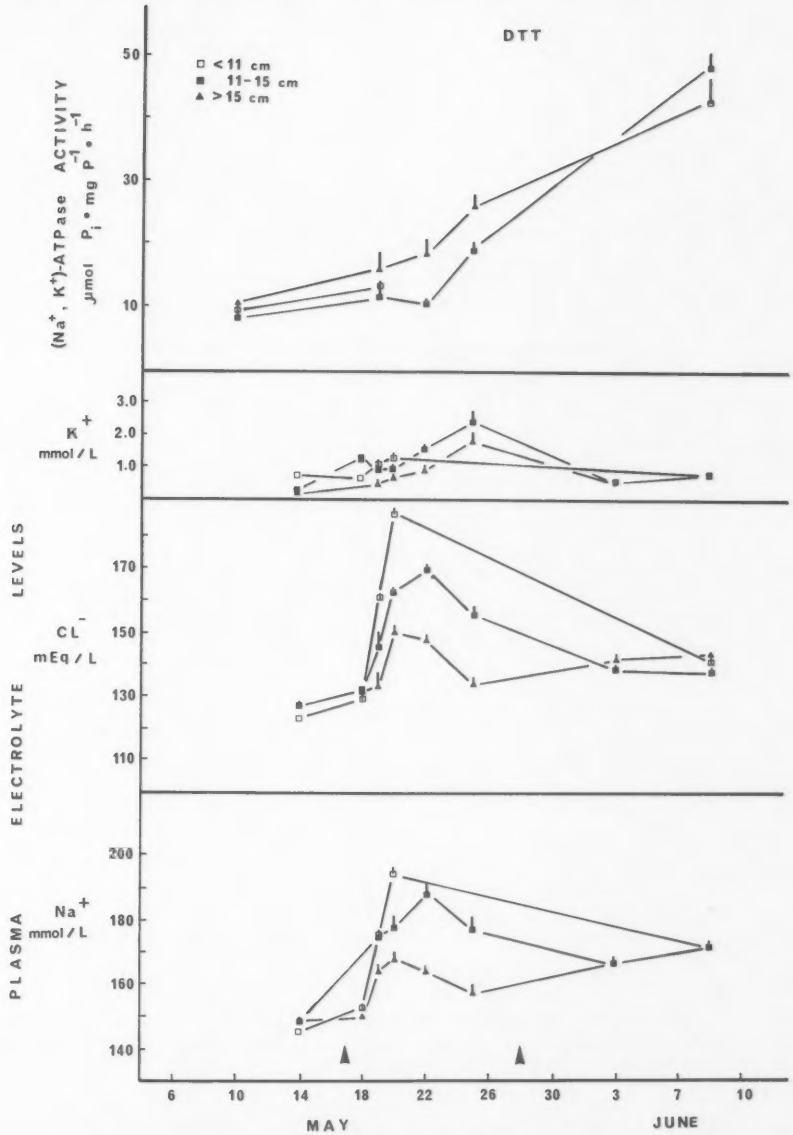


FIG. 5. Mean gill (Na^+,K^+)-ATPase activity and plasma K^+, Cl^-, and Na^+ concentrations (+0.5 SE) for each size group in the direct transfer from freshwater to 28–29‰ salinity condition (DTT). Transfer from freshwater to seawater was made on 17 May and from Tracadie to the Fortune River estuary culture site on 28 May. Arrowheads indicate transfer period.

transferred from Tracadie to UPEI a marked increase in K^+ levels occurred in all size groups. Potassium levels in small trout rose dramatically and later declined to more normal levels. Such low plasma K^+ concentrations may have caused the death of some trout in freshwater.

Gill ATPase activity in trout of all size groups of the FC regime increased steadily during the experimental period (Fig. 4). Although the ATPase levels were slightly higher for large trout, they were not significantly different ($p < 0.05$) than those of the smallest trout in any of the comparisons. Similarly, plasma concentrations of Na^+ and Cl^- were not significantly different ($p < 0.05$) following recovery from shipping stress.

Plasma ion levels in trout from the DTT regime in freshwater on 14 May were comparable with the other groups (Fig. 5). Following direct transfer to salinity of 26–28‰ on 17 May, plasma ion levels rose significantly ($p < 0.05$) in all size groups. The most marked increase occurred in Na^+ and Cl^- levels; the highest Na^+ and Cl^- levels were recorded from trout in the <11 cm group. The highest mortality also occurred in this group and only a few survived until 28 May. Na^+ and Cl^-

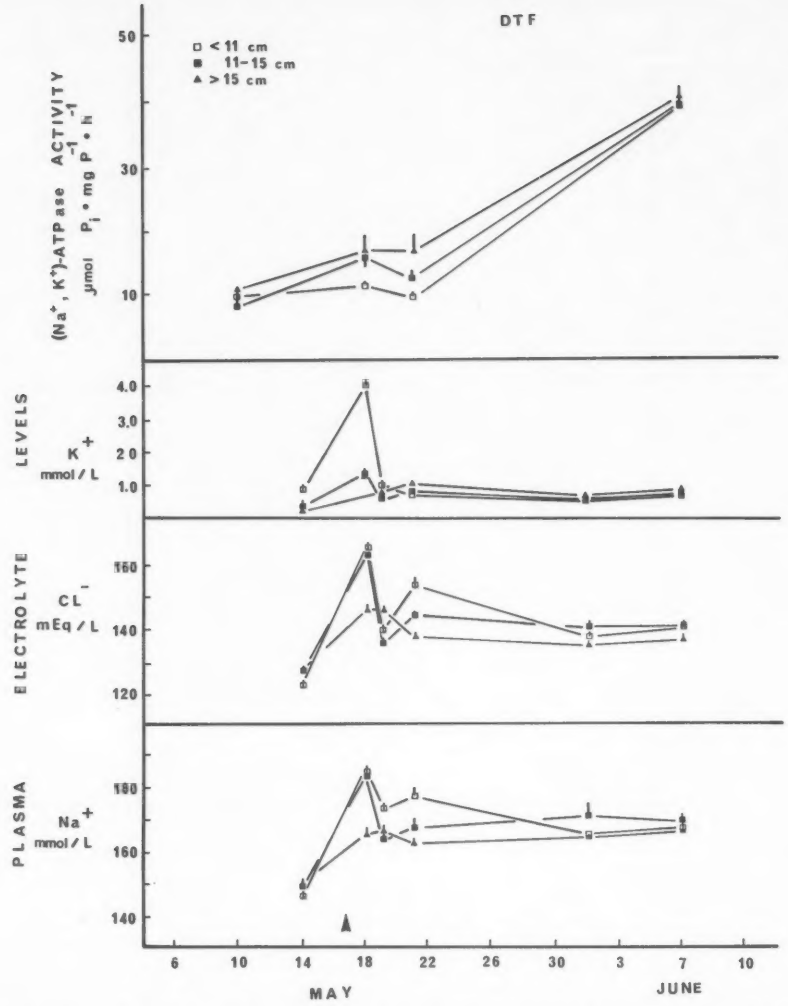


FIG. 6. Mean gill (Na^+,K^+)-ATPase activity and plasma K^+, Cl^-, and Na^+ concentrations (+0.5 SE) for each size group in the direct transfer from freshwater to seawater at Fortune Bay (DTF). Salinities at the culture site in Fortune Bay fluctuated daily from 15 to 29‰.

concentrations in trout 11–15 cm were significantly higher ($p < 0.05$) than in the larger trout. After a 7-d exposure to high salinity, trout 11–15 and >15 cm showed lower Na^+ and Cl^- levels, indicating adaptation to saline conditions. As plasma Na^+ and Cl^- levels declined on 25 May there was a significant increase ($p < 0.05$) in K^+ concentrations in medium and large trout. Transfer of trout to cages in the Fortune River estuary brought about further declines in plasma ion concentrations. Only a few small trout survived to June, and analysis of plasma ion concentrations in the survivors showed a complete adaptation to estuarine salinity conditions.

Gill ATPase activity of large trout from the DTT regime on 10 May was not significantly greater ($p < 0.05$) than the levels recorded for smaller trout. However, the activity was significantly higher ($p < 0.05$) 8 d after transfer to seawater of 28‰ salinity. In all size groups, high ATPase activity was coincident with declines in plasma Na^+ and Cl^- concentrations. Low ATPase activity was coincident with high plasma Na^+ and Cl^- levels.

On 17 May, trout in the DTF group were transferred directly from freshwater to sea cages at the Fortune River site (Fig. 6). Plasma concentrations of Na^+, K^+, and Cl^- in small and medium trout increased abruptly and significantly ($p < 0.05$) immediately following transfer. Salinities varied between 21 and 29‰ during tidal cycles. Between 21 and 25 May, salinities in the region of the cage fluctuated more widely varying from 13 to 29‰. Low salinities on the surface were caused by heavy rains and tides. Stress created by high salinity under these conditions was tolerated better by trout <11 cm than those in the DTT or SAT regime.

Trout in the SAT regime were exposed to a slowly increasing salinity regime from freshwater on 6 May to 28‰ on 28 May (Fig. 1). All size groups tolerated increases in salinity up to 20‰. As salinity increased from 20 to 28‰, trout <11 cm

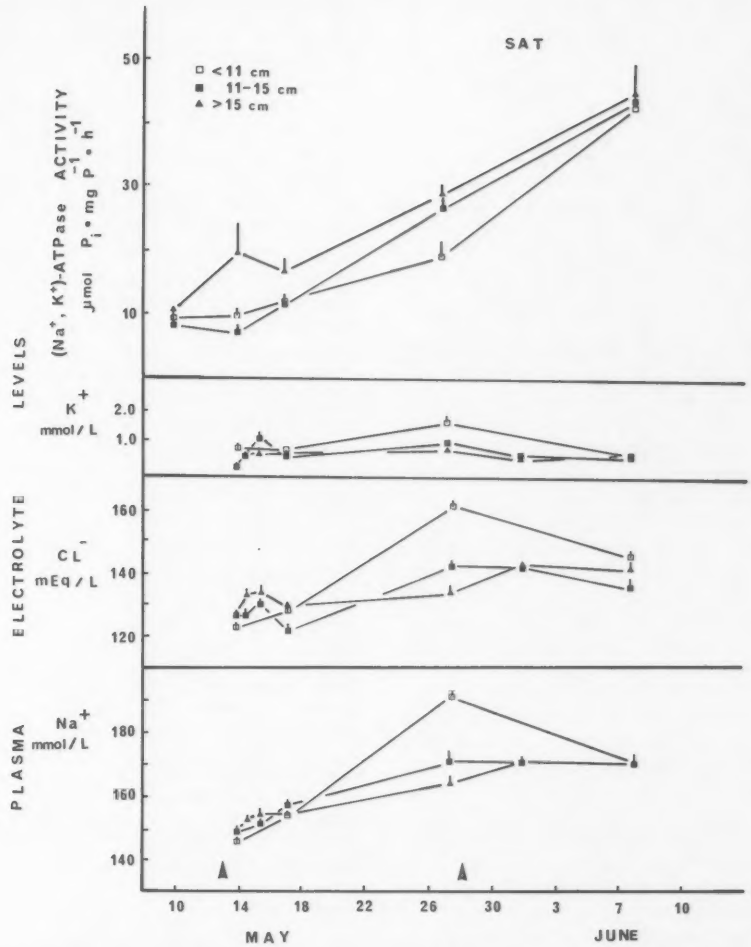


FIG. 7. Mean gill (Na^+,K^+)-ATPase activity and plasma K^+, Cl^-, and Na^+ concentrations (+0.5 SE) for each size group in the slow acclimation regime (SAT) in which salinity was increased from freshwater to 26–28‰ salinity during a 22-d period. On 28 May, all trout were transferred to cages in Fortune River estuary.

became stressed; plasma Na^+, Cl^-, and K^+ concentrations increased and mortality rose.

ATPase activity in all size groups in the SAT regime increased as salinity levels increased (Fig. 7). ATPase activity rose more quickly in trout >15 and 11–15 cm than in trout <11 cm. ATPase levels on 27 May were significantly greater ($p < 0.05$) for large and medium trout than small trout. As in the other groups, low ATPase activity coincided with high plasma Na^+ and Cl^- concentrations and poor ionoregulatory ability.

On 28 May, all trout were transferred to Fortune Bay. By June, plasma Na^+ and Cl^- concentrations for trout <11 cm were lower and were not significantly different from larger fish. ATPase activity increased in trout <11 cm and was not different from that in large trout.

Discussion

We suggest that different seawater adaptation strategies will have to be employed for coastal and estuarine sites if survival and growth of rainbow trout are to be maximized. Seawater adaptation is a distinct physiological process dependent on body size. Generally, small rainbow trout <30 g have difficulty maintaining osmotic control when transferred to salinities above 28‰ (Jackson 1981). Landless (1976) reported that 55-g rainbow trout were unaffected by a direct transfer from freshwater to 25‰; however, mortality occurred above 30‰. Johnston et al. (1983) demonstrated that rainbow trout with an initial mean fork length of 11.9 cm and weight of 17.9 g could adapt to salinities of 28‰ if salinity was increased slowly for 15–20 d. In the present experiment, direct transfer of rainbow trout from freshwater to seawater of 28‰ salinity imposed a pronounced physiological stress on trout <11 cm fork length. This salinity also caused 40% mortality in the 11–15 cm group and 17% mortality in the >15 cm group. Death was caused by an inability to regulate high plasma Na^+ and Cl^- concentrations in the first 72 h and maintain cell and plasma water.

The ability to regulate high plasma Na^+ and Cl^- concentrations appears to be determined in part by the development of ion

pumps within the chloride cells of the gill (Keys and Willmer 1932; Maetz and Bornancin 1975; Foskett and Scheffey 1981). Chloride cell size, number, membrane structure, and (Na^+,K^+)-ATPase activity increase a few days after fish are transferred to seawater (Shirai and Utida 1970; Sargent and Thomson 1974; Giles and Vanstone 1976; Boeuf et al. 1978; Folmar and Dickhoff 1980; Philpott 1980; Sardet 1980). In our study, ATPase activity increased 5 d after transfer to seawater, which is in keeping with the 4–6 d required for de novo synthesis of gill epithelial proteins (Conte and Lin 1967). From our data we suggest that trout <11 cm activate and develop branchial ion pumps more slowly than large trout. ATPase activity was significantly lower for small ($p < 0.05$) than large trout in DTT, SAT, and DTF regimes.

As branchial ATPase activity increased, plasma Na^+ and Cl^- concentrations decreased. Only 13–22 and 16–32% of the total change in ATPase activity in the SAT and DTT regime, respectively, in May and June was attributable to a seasonally determined endogenous cycle. Most of the increase in ATPase activity was in response to internal physiological conditions induced by high plasma Na^+ and Cl^- concentrations.

Failure of small rainbow trout to adapt to high salinity appears determined by the extent of rise in plasma ion levels in the first several days following exposure and by the inability to elevate (Na^+,K^+)-ATPase activity to successfully remove plasma ions. While small trout may tolerate short-term tissue dehydration and ionic interference of cellular function, gill (Na^+,K^+)-ATPase activity must increase for adaptation to occur. Failure to substantially increase ATPase activity in the long term will lead to ionic overload, dehydration, and death. It is possible that larger rainbow trout (>11 cm fork length) have a greater latent activity of the (Na^+,K^+)-ATPase enzyme system than small trout. Lower plasma Na^+ and Cl^- levels in larger trout immediately following salinity increase may represent a latent enzyme function or some other ionoregulatory mechanism not associated with this enzyme system. It is not clear from this study whether some other ionoregulatory mechanism exists or whether differences in plasma Na^+ and Cl^- levels are attributable to differences in gill surface area, membrane permeability, or drinking rates.

It may be possible to elevate ATPase activity and reduce seawater adaptation time for small trout by feeding them a diet high in salt prior to seawater transfer. Jackson (1977) concluded that adding 10% salt to the diet improved survival of rainbow trout when exposed to 30‰ seawater. Adding 12% salt to the diet of freshwater coho salmon (*Oncorhynchus kisutch*) and Atlantic salmon (*Salmo salar*) also improved survival in seawater and increased branchial (Na^+,K^+)-ATPase activity (Zaugg and McLain 1969; Basulto 1976). Further work is needed to clarify the level of salt needed in the diet to increase ATPase activity and salinity tolerance in rainbow trout of different sizes before such methods can be employed in fish farming. The direct transfer of trout to the Fortune Bay site demonstrated that trout of all sizes adapted to salinity conditions more quickly in the estuary than in any of the other conditions. Although salinities were as high as 28–29‰, salinity fluctuations during tidal cycles lowered the level and duration of osmotic and ionic stress. All trout <15 cm were initially stressed by salinity conditions following transfer, but Na^+ concentrations in the <11 cm group did not rise above 190 mmol/L, as was the case in the other regimes. Only 27% mortality occurred in small trout at Fortune Bay. Fluctuating salinities in the estuarine environment, therefore, appear to provide temporary relief from ionic stress and furnish a more suitable environment for the adaptation process. Trout from the other regimes when transferred to the Fortune Bay site also displayed better ionoregulatory control and improved growth.

There are several advantages to using estuaries for the adaptation and culture of rainbow trout. Unlike coastal areas, salinity conditions in estuaries are more favorable for transferring small trout from freshwater directly into seawater without prior exposure to intermediate salinities. Seawater temperature, accessibility, and the protection from storms provided by estuaries create more favorable rearing and growth conditions for rainbow trout than many coastal areas. In intensive fish culture operations, brackish water will also reduce the toxic effect of nitrites, which present a problem in freshwater, prevent fungus, and improve food consumption and trout growth (Crawford and Allen 1977; Perrone and Meade 1977). Adapting rainbow trout to seawater by direct transfer to sea cages situated in estuaries would significantly reduce the cost of seawater adaptations. Although direct transfer of trout to sea pens may be cost effective, the salinity–depth profiles and seasonal salinity changes should be known before the seawater adaptation process is initiated. Good health and a sustained growth rate may be ensured by selecting the most appropriate size for the salinity conditions at the culture site.

Acknowledgements

We thank Mr. T. P. Birt, Mr. A. McLennan, and Mr. N. MacNair for their assistance with the field and laboratory measurements; Mr. J. L. Murphy, Department of Fisheries and Oceans, for arranging the seawater adaptation and culture of rainbow trout at Tracadie and Fortune Bay; and Mr. S. Dixon for seawater rearing at Fortune Bay. This research was supported by grants to C. E. Johnston from the Natural Sciences and Engineering Research Council of Canada, the University of Prince Edward Island Senate Research Committee, and through a contract with the Department of Fisheries and Oceans, Atlantic Fisheries Gulf Region.

References

BASULTO, S. 1976. Induced saltwater tolerance in connection with inorganic salts in the feeding of Atlantic salmon (*Salmo salar* L.). Aquaculture 8: 45–55.

BATH, R., AND F. B. EDDY. 1979. Salt and water balance in rainbow trout (*Salmo gairdneri*) rapidly transferred from fresh to sea water. J. Exp. Biol. 83: 193–202.

BOEUF, G., P. LASSERE, AND Y. HARACHE. 1978. Osmotic adaptation of *Oncorhynchus kisutch*. Walbaum. II. Plasma osmotic and ionic variations, and gill Na^+-K^+ ATPase activity of yearling coho salmon transferred to sea water. Aquaculture 15: 35–52.

CANAGARATNAM, P. 1959. Growth of fishes in different salinities. J. Fish. Res. Board Can. 16: 121–130.

CONTE, F. P., AND D. LIN. 1967. Kinetics of cellular morphogenesis in gill epithelium during seawater adaptation of *Oncorhynchus* (Walb.). Comp. Biochem. Physiol. 23: 945–957.

CRAWFORD, R. E., AND G. H. ALLEN. 1977. Seawater inhibition of nitrite toxicity to chinook salmon. Trans. Am. Fish. Soc. 106: 105–109.

EDDY, F. B., AND R. BATH. 1979. Ionic regulation in rainbow trout (*Salmo gairdneri*) adapted to fresh water and dilute sea water. J. Exp. Biol. 83: 181–192.

FOLMAR, L. C., AND W. W. DICKHOFF. 1980. The parr–smolt transformation (smoltification) and sea water adaptation in salmonids. A review of selected literature. Aquaculture 21: 1–38.

FOSKETT, J. K., AND C. SCHEFFEY. 1981. The chloride cell: definitive identification as the salt-secretory cell in teleosts. Science (Wash., DC) 215(8): 164–166.

GILES, M. A., AND W. E. VANSTONE. 1976. Changes in ouabain-sensitive adenosine triphosphatase activity in gills of coho salmon (*Oncorhynchus kisutch*) during parr–smolt transformation. J. Fish. Res. Board Can. 33: 54–62.

HOUSTON, A. H. 1961. Influence of size upon the adaptation of steelhead trout (*Salmo gairdneri*) and chum salmon (*Oncorhynchus keta*) to sea water. J. Fish. Res. Board Can. 18: 401–415.

JACKSON, A. J. 1977. Reducing trout mortalities after sea water transfer. Fish Farming Int. 4: 31–32.

1981. Osmotic regulation in rainbow trout (*Salmo gairdneri*) following transfer to sea water. Aquaculture 24: 143–151.

JOHNSTON, C. E., T. P. BIRT, AND J. L. MURPHY. 1983. Branchial sodium-potassium ATPase activity and growth of rainbow trout in sea water. Prog. Fish-Cult. 45(1): 19–23.

KEYS, A., AND E. N. WILLMER. 1932. Chloride secreting cells in the gills of fishes with special reference to the common eel. J. Physiol. (Lond.) 76: 368–378.

LANDLESS, P. 1976. Acclimation of rainbow trout to sea water. Aquaculture 7: 173–179.

MAETZ, J., AND M. BORNANCIN. 1975. Biochemical and biophysical aspects of salt secretion by chloride cells in teleosts. Fortschr. Zool. 23: 322–362.

PERRONE, S. J., AND T. L. MEADE. 1977. Protective effect of chloride on nitrite toxicity to coho salmon (*Oncorhynchus kisutch*) J. Fish. Res. Board Can. 34: 486–492.

PHILPOTT, C. W. 1980. Tubular system membranes of teleost chloride cells: osmotic response and transport sites. Am. J. Physiol. 238: 171–184.

SARDET, C. 1980. Freeze fracture of the gill epithelium of euryhaline teleost fish. Am. J. Physiol. 238: 207–212.

SARGENT, J. R., AND A. J. THOMSON. 1974. The nature and properties of the inducible sodium plus potassium ion dependent adenosine triphosphatase in gills of eels (*Anguilla anquilla*) adapted to fresh water and seawater. Biochem. J. 144: 69–75.

SEDGWICK, S. D. 1970. Rainbow trout farming in Scotland. Farming trout in salt water. Scott. Agric. 49: 180–185.

SHIRAI, N., AND S. UTIDA. 1970. Development and degeneration of the chloride cell during sea water and fresh water adaption of the Japanese eel *Anguilla japonica*. Z. Zellforsch. Mikrosk. Anat. 103: 247–264.

ZAUGG, W. S., AND L. R. MCLAIN. 1969. Inorganic salt effects on growth, salt water adaption and gill ATPase of Pacific salmon, p. 293–306. *In* O. W. Neuhaus and J. E. Halver [ed.] Fish in research. Academic Press, New York, NY.

Physiological Response of Juvenile Rainbow Trout, *Salmo gairdneri*, to Acid and Aluminum — Prediction of Field Responses from Laboratory Data

C. M. Neville

Ontario Ministry of the Environment, Water Resources Branch, Aquatic Contaminants Section, Aquatic Toxicity Unit, P.O. Box 213, Rexdale, Ont. M9W 5L1

Neville, C. M. 1985. Physiological response of juvenile rainbow trout, *Salmo gairdneri*, to acid and aluminum — prediction of field responses from laboratory data. Can. J. Fish. Aquat. Sci. 42: 2004–2019.

An initial 1- to 2-h exposure of juvenile rainbow trout (*Salmo gairdneri*) to 2.8 μ*M* (= $\mu mol \cdot L^{-1}$) inorganic aluminum caused increased ventilation and activity responses ranging from slight to severe depending on the ambient pH. The severity of physiological response after 6–11 d of exposure was similar to that of the initial response. This was severe at pH 6.1, 4.5, and 4.0, slight to moderate at pH 5.0 and 5.5, but minimal at pH 6.5. In the severe response groups, 40–90% of the fish died after a few days of exposure. Death was due primarily to hypoxia at pH 6.1 and to electrolyte loss at pH 4.5 and 4.0. Between pH 5.5 and 5.0 there was a transition between the two mechanisms. With 8–10 mg natural organic acids $\cdot L^{-1}$ the response to the same concentration of inorganic aluminum was unchanged at pH 5.0 but was slightly increased at pH 4.5. Fish kills of salmonids that have occurred in South Norway could have been predicted from these results. In North America the presence of refuge areas and the prevalence of more tolerant species could account for the occurrence of sublethal rather than lethal responses.

Des truites arc-en-ciel (*Salmo gairdneri*) juvéniles exposées pour la première fois pendant 1 à 2 h à une concentration de 2,8 μ*M* (= $\mu mol \cdot L^{-1}$) d'aluminium inorganique ont présenté une augmentation de la ventilation et de l'activité allant de légère à très importante, selon le pH ambiant. L'importance de la réponse physiologique notée après de 6 à 11 jours d'exposition était semblable à celle de la réponse initiale. Elle était très importante aux pH 6,1, 4,5 et 4,0, de légère à modérée aux pH 5,0 et 5,5 et très faible au pH 6,5. De 40 à 90 % des poissons des groupes à réponse très importante sont morts après quelques jours d'exposition. La mort était surtout causée par hypoxie à un pH 6,1 et à la perte d'électrolytes aux pH 4,5 et 4,0. On notait une transition entre ces deux mécanismes dans la gamme des pH de 5,0 et 5,5. La présence de 8 à 10 $mg \cdot L^{-1}$ d'acides organiques naturels n'a pas modifié la réponse à la même concentration d'aluminium inorganique à un pH 5,0, mais celle-ci était légèrement accrue à un pH 4,5. Les hécatombes de salmonidés survenues dans le sud de la Norvège auraient pu être prévues à partir de ces résultats. En Amérique du Nord, l'existence de zones refuges et d'espèces plus tolérantes pourrait expliquer pourquoi les réponses obtenues sont plutôt sublétales que létales.

Received January 31, 1985
Accepted August 20, 1985
(J8095)

Reçu le 31 janvier 1985
Accepté le 20 août 1985

Effects of acid precipitation on aquatic environments have been the subject of intensive studies for over a decade in Scandinavia and North America (reviews in Harvey et al. 1981; Haines 1981; Dillon et al. 1984). The physiological impact on fish is reviewed by Fromm (1980) and Booth et al. (1982). Fish populations have been lost from many lakes and streams sensitive to acidification (Beamish and Harvey 1972; Jensen and Snekvik 1972; Beamish 1974; Wright and Snekvik 1978), and Schofield (1976), Schofield and Trojnar (1980), and Muniz and Leivestad (1980a) showed that the losses often occurred at pH values higher than those predicted by laboratory studies.

The toxicity of acidic environments was found to be greatest at low calcium concentrations (Wright and Snekvik 1978). However, high aluminum concentrations were suggested as being the main toxic agent (Dickson 1978; Cronan and Schofield 1979; Schofield and Trojnar 1980), since aluminum is leached from siliceous soils and bedrock by acidification, and high concentrations accompany the acidic input to lakes and streams during snowmelt. Subsequently, it was shown that only the inorganic labile species of aluminum were toxic (Driscoll et al. 1980; Baker and Schofield 1980). Previous assessments of toxicity using total levels of aluminum were misleading, as organic aluminum species often form a major part of the total aluminum. Baker and Schofield (1980) found that aluminum plus citric acid (a low molecular weight organic acid) was not toxic to brook trout (*Salvelinus fontinalis*) fry at pH 5.2 and 4.4. However, neither the toxicity of natural organic aluminum species nor the influence such species may have on the toxicity of labile inorganic aluminum species has been investigated.

Howells et al. (1983) reviewed the effects of acid, calcium, and aluminum on fish survival and predicted that at pH 4.5 and

calcium levels less than 0.8 mg·L^{-1}, many lakes will not support fish. However, they were not able to include aluminum in their model because of the lack of suitable biological data.

Most studies on aluminum toxicity have been done on the early life stages of various species, as recruitment failure is one of the most common causes of population decline. Yolk sac larvae and fry have been found by many workers to be more sensitive to aluminum toxicity than the eggs (Schofield and Trojnar 1980; Baker and Schofield 1980; Holtze 1983). However, some population structures studied by test fishing have shown responses of brown trout (*Salmo trutta*) (Muniz and Leivestad 1980a) and brook trout (Frenette and Dodson 1984) to be different from those expected if recruitment failure were the only cause of population decline. This was also shown in laboratory studies where presmolt Atlantic salmon (*Salmo salar*) (Rosseland and Skogheim 1982) and 1-yr-old brown trout (Brown 1983) were found to be more sensitive than their respective early life stages.

Nevertheless, there are only a few reports of fish kills in lakes and streams which have been affected by acid precipitation (Leivestad and Muniz 1976; Schofield and Trojnar 1980; Farmer et al. 1980; Grahn 1980; Rosseland and Skogheim 1982; Henriksen et al. 1984). Concentrations of inorganic monomeric aluminum that occur in the natural environment are more likely to produce sublethal stress than to be directly lethal. Under sublethal conditions it has been shown that there is a greater than normal energy expenditure for maintenance of homeostasis, thus reducing energy levels available for growth and reproduction (Muniz and Leivestad 1980a; Rosseland 1980).

The present study was therefore carried out using juvenile rainbow trout (*Salmo gairdneri*) primarily to investigate the physiological response to sublethal concentrations of labile inorganic aluminum at various pH levels in a low-ion environment. The pH and other ion concentrations used were based on the concentrations found in acidified softwater lakes and streams in the Muskoka–Haliburton area of Ontario. The tests were done with and without the presence of organic aluminum so that it was possible to extrapolate laboratory data to predict the effects of similar conditions in the environment on juvenile and adult salmonids. The predictions were validated by comparison with reported fish kills in the field.

Materials and Methods

Fish Preparation

Rainbow trout (197–469 g) were obtained from Aquafarms Ltd., Feversham, Ontario, and held in dechlorinated Toronto tap water (pH = 7.5, Ca = 1 m*M* (*M* = mol·L^{-1}) at 10 ± 1°C. They were fed EWOS trout pellets (Rundle Feed Mills) daily until 48 h before being cannulated. After acclimation to soft water (prepared by reverse osmosis from dechlorinated Toronto tap water) at pH 6.1–6.3 for approximately 10 d, the dorsal aorta of the fish was cannulated following the procedure of Soivio et al. (1972) for blood sampling on the last day of exposure. They were placed in individual, clear, plexiglas respirometers for a 24-h recovery period at pH 6.1–6.3 before being exposed to the test medium. Water temperature was controlled by partially submerging the respirometers in a 10 ± 2°C waterbath. The respirometers were kept in an area shielded by curtains to protect them from strong direct light and to minimize stress caused by extraneous activity. The fish were "at rest" during the recovery period and the experiments.

Experimental Protocol

Each experiment consisted of two groups of six fish exposed to the same pH. An "acid" group served as a control for an "acid + aluminum" group to find the effect of labile inorganic aluminum at the various pH levels under investigation. Most experiments were carried out using a flowthrough exposure system. In preliminary experiments at pH 5.5–6.3, fish survived less than 24 h with an inorganic labile aluminum concentration of 6.8 μ*M* and 48–72 h at 3.8 μ*M*, but at 1.8 μ*M* they showed little change after 8 d of exposure. A sublethal aluminum concentration of 2.8 μ*M* (75 μg·L^{-1}) was therefore chosen. pH levels investigated were 4.0, 4.5, 5.0, 6.1, and 6.5. There were two replicates at each pH level except at pH 6.5. Exposure time was 11 d in the flowthrough experiments except at pH 6.1, 4.5, and 4.0 in which some fish died after a few days of exposure (see Table 1). Subsequently, in those experiments fish were killed before the end of the exposure period when their pattern of ventilation rate and oxygen consumption indicated that they were no longer able to take up enough oxygen from the water. Thus, variables were measured on these fish before they became moribund. One experiment was also carried out at pH 6.1 during two cycles of intermittent (24 h on, 24 h off) exposure to 2.8 μ*M* aluminum, and a low aluminum experiment was carried out at pH 6.1 using 1.6 μ*M* (45 μg·L^{-1}) aluminum.

To determine the effect of organic aluminum on inorganic aluminum toxicity, an "acid + aluminum" group was compared with an "organic acid + aluminum" group at pH 4.5 and 5.0 during a 6-d exposure period. Both groups were acidified to the test pH using sulphuric acid and had approximately the same concentration of labile inorganic aluminum in the exposure medium. One experiment was carried out at pH 4.5 + organic acid without any additional aluminum as a control for the effect of organic aluminum, and a positive control experiment at pH 4.5 was run using a "metal-free" fulvic acid preparation (Department of Chemistry, Concordia University) plus aluminum. This experiment was used as a heavy metal control to see if any toxic reaction during the 6-d exposure period could be attributed to the heavy metals present in natural organic acid instead of the labile inorganic aluminum. If so, the toxicity of the heavy metal free fulvic acid + aluminum group would be less than that of the natural organic acid + aluminum group. Acidic stream water with a high concentration of natural organic acids (from Dickie Lake Inlet No. 11, Muskoka) was transported to the laboratory in Toronto for these experiments. This limited the supply of water, so a recirculating system with 90% replacement every 24 h had to be used instead of a flowthrough system. Hence, there was time for some of the aluminum to form small polymers instead of all being monomeric. A control experiment for this exposure system was run at pH 5.5 using an "acid only" group with an "acid + aluminum" group for comparison.

Exposure Systems

During the flowthrough experiments, dechlorinated Toronto tap water, softened by reverse osmosis, was acidified with sulphuric acid to the required pH ± 0.2 unit using a Radiometer pH stat system. After being well mixed and degassed it was allowed to flow from a head tank by gravity to two flowsplitters and then through each of six respirometers per flowsplitter at a rate of 500 mL·min^{-1}. Aluminum sulphate ($Al_2(SO_4)_3 \cdot 18H_2O$, 28 m*M* stock solution) was added to the acidified water before it entered one of the flowsplitters, using a peristaltic pump at a rate

TABLE 1. Concentrations of labile inorganic aluminum, labile organic aluminum, and calcium in the exposure medium and the exposure period of the fish in each group.

Group[a]	Inorganic aluminum, $\mu g \cdot L^{-1}$ (μM)	Organic aluminum, $\mu g \cdot L^{-1}$ (μM)	Ca^{2+}, $mg \cdot L^{-1}$ (μM)	Days of fish exposure[b]
6.5 Ac(C)	3 (0.1)	—	4.8–5.0 (120)	11
6.1 Ac(C)	3 (0.1)	—	2.7–2.9 (70)	11
5.5 Ac(C)[c]	3 (0.1)	—	2.1–2.5 (60)	6
6.5 Al	75±10 (2.8)	—	4.8–5.0 (120)	11
6.1 Al	75±10 (2.8)	—	2.7–2.9 (70)	4, 6(D), 4(K), 3 × 11
6.1 Al	75±10 (2.8)	—	5.3–6.1 (140)	4 × 2, 3(D), 3(K)
6.1 Al(L)	45±10 (1.7)	—	5.3–6.1 (140)	11
6.1 Al(L)	45±10 (1.7)	—	2.7–3.1 (70)	7, 9, 4 × 11
5.5 Al[c]	70±10 (2.6)	—	2.1–2.7 (60)	6
5.0 Ac	3 (0.1)	—	3.9–4.1 (100)	11
5.0 Ac	3 (0.1)	—	3.3–3.5 (85)	11
5.0 Al	75±10 (2.8)	—	3.3–3.5 (85)	11
5.0 Al[c]	70±15 (2.6)	—	1.8–2.5 (55)	6
5.0 Org Al[c]	90±15 (3.3)[d]	275±20 (10.2)	2.1–3.3 (50–80)	6
4.5 Ac	3 (0.1)	—	2.4–2.6 (60)	11
4.5 Ac	3 (0.1)	—	3.0–3.5 (80)	10
4.5 Al	75±10 (2.8)	—	2.4–2.6 (60)	2 × 7, 8(D), 6, 7(K), 11
4.5 Al	75±10 (2.8)	—	3.0–3.5 (80)	7(D), 6, 2 × 7(K), 2 × 10
4.5 Al[c]	70±15 (2.6)	—	2.8–3.1 (75)	4, 5(D), 4 × 6
4.5 Al[c]	70±15 (2.6)	—	2.9–3.8 (70–95)	6
4.5 Al[c]	75±5 (2.8)	—	3.1–4.6 (80–115)	5(D), 5 × 6
4.5 Org Al[c]	90±15 (3.3)[d]	240±20 (8.9)	2.2–3.0 (55–75)	2, 4(D), 4 × 6
4.5 (fulv.) Al[c]	80±15 (3.0)[d]	340±20 (12.6)	2.5–3.3 (60–80)	3 × 3(D), 3 × 6
4.5 Org Ac[c]	11±8 (0.4)[d]	95±10 (3.5)	2.9–3.5 (80)	6
4.0 Ac	3 (0.1)	—	2.0–2.5 (55)	1, 2(D), 2 × 1, 2, 3(K)
4.0 Ac	3 (0.1)	—	3.5–3.8 (90)	1, 2 × 2, 7(D), 2(K)
4.0 Al	75±10 (2.8)	—	2.0–2.5 (55)	4, 5, 6(D), 2 × 5, 7(K)
4.0 Al	75±10 (2.8)	—	3.5–3.8 (90)	2 × 7(K), 4 × 11

[a]C = control; Ac = acid only; Al = 75 $\mu g \cdot L^{-1}$ (2.8 μM) inorganic aluminum; Al(L) = 45 $\mu g \cdot L^{-1}$ (1.6 μM) aluminum; Org Al = +organic aluminum.

[b]A series of numbers followed by (D) or (K) indicates days of exposure of fish that died or were killed during the exposure period.

[c]Recirculation exposure system used.

[d]Estimated inorganic aluminum concentrations.

that would produce a final aluminum concentration of 2.8 μM. Washout time of the respirometers was approximately 20 min. The system ensured that any organic aluminum formed from aluminum ions combining with mucous and excretory products from the fish would be minimal and that the inorganic aluminum would not have time to form large, nonlabile polymers. Thus, total aluminum was essentially equal to labile inorganic aluminum. Ambient water pH was monitored continually throughout the experiment. Water temperature, P_{O_2}, hardness (as $CaCO_3$), and labile inorganic aluminum concentrations were measured daily, ion and metal concentrations (including total aluminum) were measured biweekly,*[1] and P_{CO_2} was measured during and after the initial pH change using a Radiometer P_{CO_2} electrode and blood gas monitor (PHM73). Aluminum, ion, and metal concentrations were measured by the Laboratory Services Branch, Ontario Ministry of the Environment, and other parameters by the Toxicity Unit. The aluminum and calcium concentrations are included in Table 1.

The recirculation system consisted of two aerated, pH- and temperature-controlled head tanks. From each tank the appropriate exposure medium flowed by gravity at a rate of 500 $mL \cdot min^{-1}$ through a flowsplitter to six respirometers and was collected in a reservoir before being pumped back to the head tank.

Prior to using the Dickie 11 stream water in the experiments, a sample was diluted with reverse osmosis water to give a dissolved organic carbon (DOC) concentration of approximately 10 $mg \cdot L^{-1}$. The pH was adjusted with sulphuric acid to the required pH, and a range of concentrations of $Al_2(SO_4)_3 \cdot 18H_2O$ were added to find the appropriate concentration of aluminum needed for each experimental situation. There had to be enough aluminum to saturate the organic acids sufficiently to leave an excess of 2.8 μM in the labile inorganic form. Aluminum speciation was measured by the Laboratory Services Branch of the Ministry of the Environment using methylisobutylketone (MIBK) for extraction of total and organic labile aluminum species. The latter was obtained from samples in which the labile and nonlabile organic aluminum had been separated from inorganic aluminum species by cation exchange (modified from Driscoll 1984). Following extraction, total and organic labile aluminum concentrations were measured by graphite furnace atomic absorption spectroscopy (GFAAS). Labile inorganic aluminum was calculated by difference.

[1]Here and throughout, an asterisk indicates that the variable is included in a complete set of tabular data available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada, K1A 0S2.

Ninety litres of the exposure medium for each group was made up daily. Appropriate volumes of aluminum sulphate were added from stock solutions to acidified, reverse osmosis water with or without Dickie 11 stream water to ensure that each medium had equal concentrations of labile inorganic aluminum. The media were well aerated to ensure complete mixing and removal of any excess carbon dioxide before being used to replace 90% of each exposure medium.

Water samples were taken from the exposure system immediately before the water change for total ammonia concentration, and immediately before and after the water change for aluminum speciation (see Table 1) and ion and metal contents (Laboratory Services Branch, Ministry of the Environment). The P_{O_2} and P_{CO_2} were measured during daily estimations of oxygen consumption levels (see below).

Variables Measured

During the initial 1–2 h of exposure in the flowthrough experiments, ventilation and cough rates,* an indication of ventilation amplitude made by estimating the gape of the mouth (millimetres) during each ventilation cycle, the degree of activity of the fish (see below), and the rate of oxygen consumption were measured. It was only possible to make observations of the time of onset, duration, and severity of response of the first four parameters during the initial exposure period of the recirculation experiments because extraneous activity during the water change precluded making exact measurements of these data. All parameters were then measured daily in all experiments during the rest of the exposure period.

The activity of the fish was estimated on a scale of 0–5 as follows:

0, barely visible ventilation, no other movement.
1, normal ventilation, very little body movement.
2, moderate amount of body movement.
3, occasional to frequent strong movement of body and fins.
4, constant strong movement of body and fins.
5, violent struggling.

At the end of the exposure period, arterial blood samples were taken for measurement of pH (Radiometer microelectrode unit E5012a and pH/blood gas monitor PHM73), oxygen content* and capacity (Tucker 1967), using a modified glass cuvette and a Radiometer P_{O_2} electrode (E5046/0), hemoglobin* (American Optical Hemoglobinemeter), lactate (Sigma Chemical Co., Procedure No. 826-UV), and plasma total CO_2* (Harleco micro CO_2 apparatus). Oxygen capacity was found by measuring the oxygen content after tonometering the blood with humidified air at 10°C for 5 min. The percent oxygen saturation was calculated from the oxygen content and capacity. Plasma P_{CO_2} was calculated from the pH and total CO_2 using the Henderson–Hasselbalch equation, with values for pK_1 and αCO_2 from Albers (1970). Further arterial blood samples were collected in unheparinized tuberculin syringes for electrolyte analysis. The blood was centrifuged within a few minutes of sampling and the plasma was immediately separated. Duplicate samples were analyzed for sodium, potassium,* calcium,* and magnesium* ions by atomic absorption and for chloride ions by ion chromatography. In a few fish where it was not possible to obtain arterial blood, venous blood was taken from the caudal vein for measurement of plasma electrolytes (except potassium) and total CO_2 and lactate estimations.

After stunning the fish, duplicate 1-g samples of epaxial muscle were taken into weighed scintillation vials for estimation of tissue water and for tissue chloride, sodium, potassium,* calcium,* and magnesium* ions (Neville 1979). Electrolytes were measured as above. Tissue samples were also taken from the gills, liver, intestine, gonads, and kidney and dried at 105°C for 48 h for aluminum analysis. In the recirculation experiments, gill and a few kidney tissue samples were taken immediately after stunning the fish for electron microscopy examination (J. H. Youson and C. M. Neville, in prep.).

Data Analysis

Data were analyzed using a one-way analysis of variance for comparisons between group parameters. No significant difference was computed if the means of both groups were within the normal range and if the range of the individual data in each group was within limits determined by the accuracy of the method used for the parameter estimation. Data from the flowthrough and recirculation systems were compared to ensure the validity of combining data (see Appendix). Acid only groups exposed at pH 6.5, 6.1 (flowthrough system), and 5.5 (recirculation system) were combined to form the control group.

Data Reporting

The mean, standard error, and significance ($P < 0.05$) of major parameters measured at the end of the exposure period are shown as histograms in Fig. 4–7.

Results

In all of the experiments, ambient P_{O_2} and P_{CO_2} remained at normal levels ($P_{O_2} \approx$ 140–150 mmHg, $P_{CO_2} <$ 1 mmHg, 1 mmHg = 133.322 Pa) throughout the exposure period, and although ambient total ammonia concentrations rose to almost 3.0 $mg \cdot L^{-1}$ after 24 h in the recirculation system, the unionized ammonia concentration ($<30\ \mu g \cdot L^{-1}$ at pH 4.5–5.5) was less than 10% of chronic toxicity levels (Smart 1976). Hence, ventilatory and activity reactions by the fish were not due to ambient oxygen, carbon dioxide, or ammonia levels.

Table 2 shows the initial response to the exposure medium (i.e. during the first 1–2 h of exposure). The group responses are categorized as nil, slight, moderate, or severe depending on the extent and duration of changes in activity, ventilation rate, ventilation amplitude, or cough rate.

Acid only groups showed no response at pH 6.5–5.5 (controls) or at pH 5.0, but there was a moderate response at pH 4.5 and a very severe response at pH 4.0. With 2.8 μM inorganic aluminum the response was slight at pH 6.5, moderate at pH 5.5 and 5.0, and severe at pH 6.1, 4.5, and 4.0. However, the low inorganic aluminum (1.6 μM) group at pH 6.1 showed no response. There was no response in the organic acid control group at pH 4.5, and in the presence of organic aluminum at pH 4.5 and 5.0 the response to inorganic aluminum was reduced. In all groups showing an initial reaction to the exposure medium, values returned to preexposure levels after 2–3 h of exposure.

An outline of the subsequent changes in activity and ventilation parameters during the 6- to 11-d exposure period is shown in Fig. 1–3, based on the means and standard errors (shaded) of daily values. Activity remained at or near control values throughout the experiments except for a few brief periods of struggling in the pH 5.0 acid only group and pH 5.0 and 4.5 acid + inorganic aluminum groups during days 3–7, and very low activity levels in all groups exposed to organic acids. Ventilation responses seen during the initial exposure period gradually

TABLE 2. Activity, ventilation, and cough rate responses of the fish to the test conditions during the first 1–2 h of exposure (see Table 1 for explanation of groups).

Group	Severity and approximate times of responses	Category of initial response
Controls (pH 6.5–5.5)	No initial response	Nil
6.5 Al	Slight increase in ventilation rate after 1 h	Slight
6.1 Al	Severe increase in activity and ventilation parameters after 15 min Moderate increase in cough rate after 1 h	Severe
6.1 Al (L)	No initial response	Nil
5.5 Al	Moderate transient increase in all parameters after 15 min	Moderate
5.0 Ac	No initial response	Nil
5.0 Al	Moderate transient increase in activity after 5 min	Moderate
5.0 Org Al	Slight transient increase in activity after 5 min	Slight
4.5 Ac	Moderate increase in ventilation parameters and cough rate after 5 min for 1 h	Moderate
4.5 Al	Severe increase in activity and ventilation parameters from 5 min for 10 min Moderate increase in activity and ventilation parameters from 15 min for 1 h Slight increase in cough rate after 1 h	Severe
4.5 Org Al	Severe increase in activity and ventilation parameters from 10 min for 10 min	Moderate to severe
4.5 Org Ac	No initial response	Nil
4.0 Ac	Moderate increase in activity from <5 min for 5 min Severe increase in ventilation parameters and cough rate from <5 min for at least 1–2 h	Severe
4.0 Al	Slight to moderate increase in activity from 5 min for 5 min Moderate to severe increase in ventilation parameters from 5 min for at least 1–2 h Moderate increase in cough rate from 1 h for at least 1 h	Severe

redeveloped during the following days after more prolonged exposure but were not activity related. Groups with little or no initial response continued to show little change except for a short 2-d increase in ventilation and cough rates in the pH 6.5 + aluminum and pH 6.1 + low aluminum groups. Most of the moderate and severe response groups showed increased ventilation amplitude as well as ventilation and cough rates, even though activity levels were low. Oxygen consumption rates were usually high for a few days but decreased as the fish became more severely stressed. In the severe response groups, despite increased ventilation rates and amplitudes, some of the fish died (or had to be killed) before the end of the exposure period. Ninety percent of the acid only group at pH 4.0 died in 1–3 d, whilst with inorganic aluminum, 75% of the fish at pH 6.1 died in 2–4 d, 90% at pH 4.5 died in 4–8 d, and 66% at pH 4.0 died in 4–7 d. With organic as well as inorganic aluminum at pH 4.5, 40% died within 4 d.

Ventilation parameters of fish intermittently exposed to aluminum at pH 6.1 for 4 d fluctuated in close correlation with the ambient aluminum concentration (Fig. 1b). The responses were similar to those in the constant exposure group during aluminum exposure but normal values were restored during the first aluminum-free period. Response to the second exposure period was slightly less than to the first. Similarly, recovery was not complete during the second aluminum-free period.

Figures 4 and 7 (row A) show the significant differences in activity and ventilation parameters between the experimental groups and the controls on the last day of exposure. Results were similar to those during the exposure period. The histograms also include the level of gill aluminum accumulation. In the inorganic aluminum groups this was the highest at pH 6.1, high at pH 6.5, moderate at pH 4.5, 5.0, and 5.5, but only slight at pH 4.0. Compared with the acid + aluminum groups, with organic acids the gill aluminum level was halved at pH 4.5 and slightly reduced at pH 5.0. In the low aluminum group at pH 6.1, gill aluminum accumulation was moderate. (Histograms of this

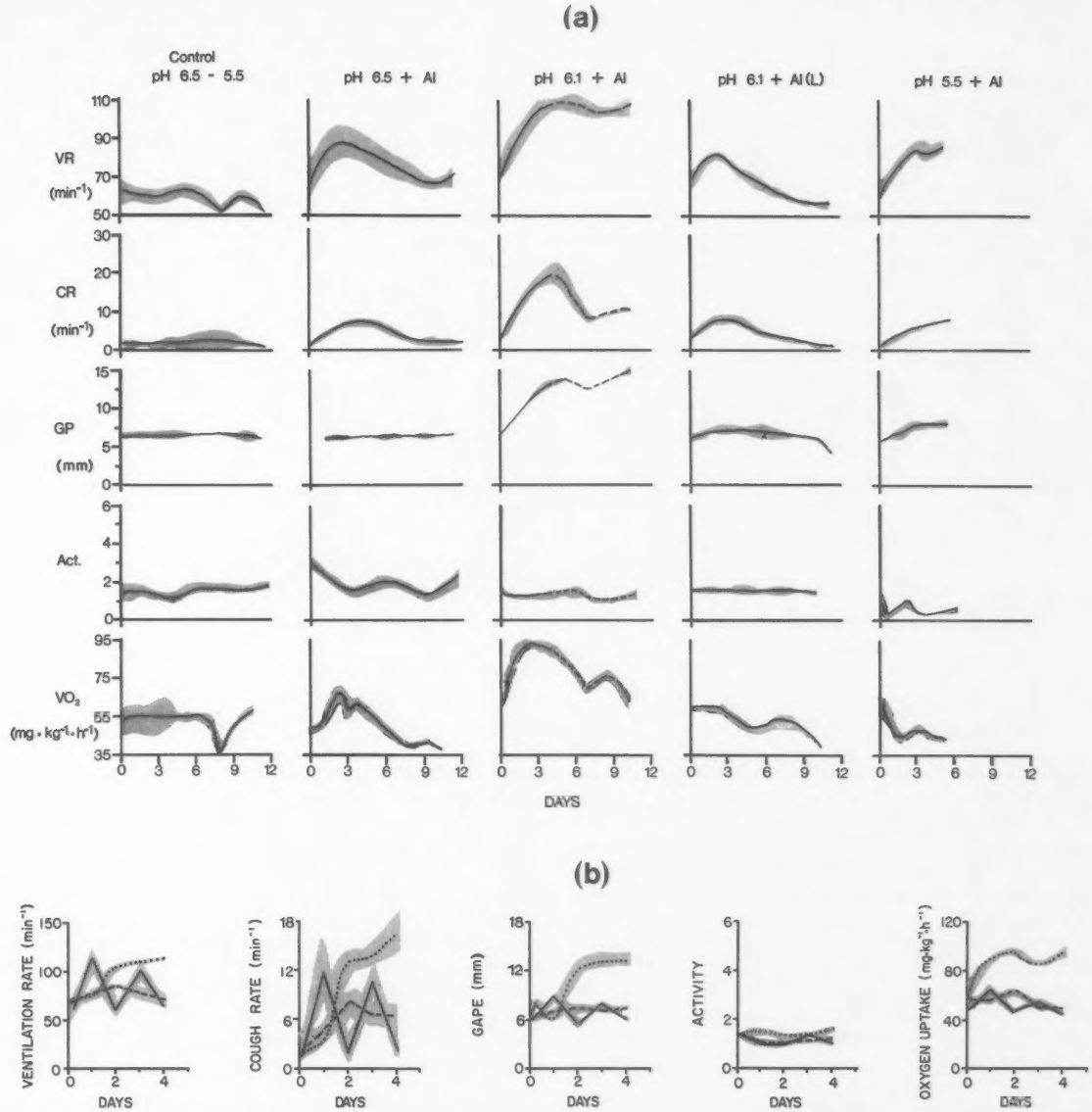


FIG. 1. (a) Ventilatory and activity responses throughout the exposure period at pH 6.5–5.5 (---, 25% of fish only). Al = 2.8 μ*M* labile inorganic aluminum; Al(L) = 1.6 μ*M* labile inorganic aluminum; VR = ventilation rate; CR = cough rate; GP = gape; Act. = activity (1 = normal fin and ventilation movements of unstressed, resting fish; 5 = severe struggling); VO_2= oxygen uptake rate. (b) Ventilatory and activity responses during 4 d of intermittent exposure to pH 6.1 + 2.8 μ*M* labile inorganic aluminum (—) compared with continuous exposure to 2.8 μ*M* (···) and 1.6 μ*M* (---) aluminum.

group have not been included in Fig. 4, 5, 6, as no other parameters were significantly different from the controls at the end of the 11-d exposure period.)

In the oxygen transport and acid–base balance histograms (Fig. 5 and 7, row B) levels of haemoglobin, oxygen content, and total CO_2 are reflected in the oxygen capacity, oxygen saturation, and hydrogen ion concentration plus carbon dioxide tension, respectively. Moderate to severe acidaemia (H^+) occurred in all the organic acid groups and the inorganic aluminum groups at pH 5.0, 4.5, and 4.0. Slight but significant acidaemia occurred at pH 6.1 and 5.5 with aluminum and pH 4.5 and 4.0 without aluminum. The acidaemia was caused primarily by retention of carbon dioxide (P_{CO_2}) in the organic acid groups and the pH 5.0 + aluminum group and by lactic acid in the pH 6.1 + aluminum and pH 4.0 and 4.5 acid only groups, but neither lactate nor P_{CO_2} were significantly increased in the inorganic aluminum groups at pH 4.5 and 4.0.

Haemoglobin levels were significantly increased in the more acidaemic groups, resulting in higher levels of arterial blood oxygen capacity. Increased ventilation rates resulted in the arterial blood oxygen saturation levels being slightly but not significantly below the control values of 91%. However, in the

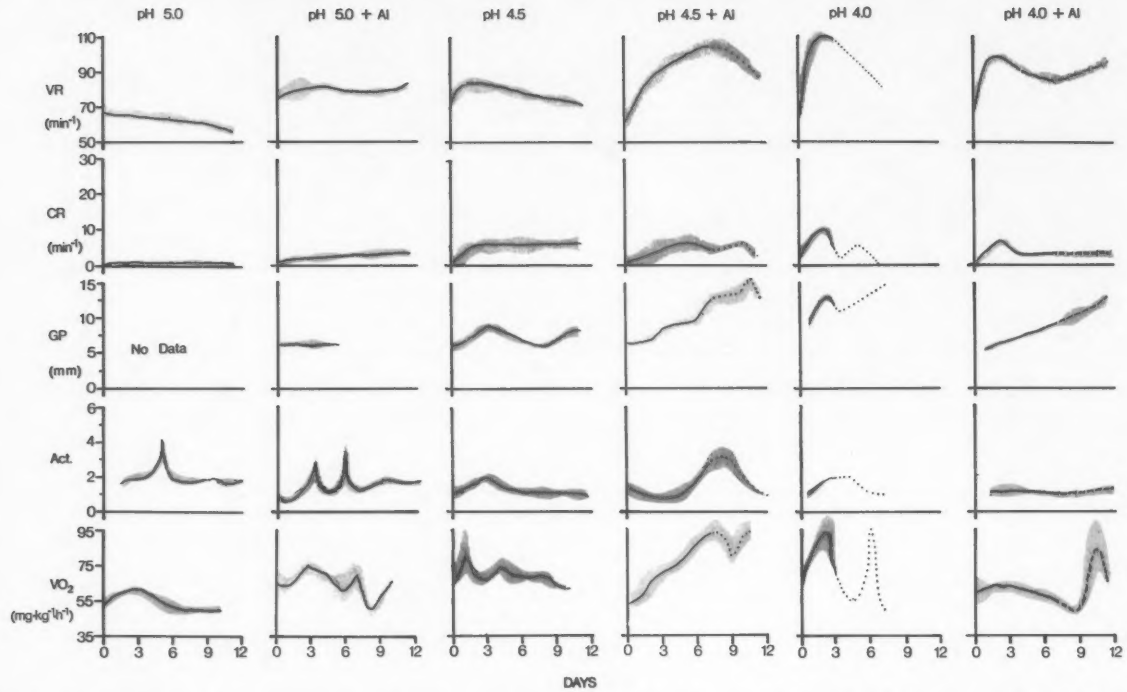


FIG. 2. Ventilatory and activity responses throughout the exposure period at pH 5.0, 4.5, and 4.0 (···, 10% of fish only; ---, 33% of fish only). Al = 2.8 μ*M* labile inorganic aluminum. See Fig. 1 for definition of parameters on *y*-axis.

pH 4.0 acid only and pH 6.1 + aluminum groups, arterial blood oxygen saturation levels were only 73 and 43%, respectively.

The electrolyte histograms (Fig. 6 and 7, row C) include only the extracellular ions, since changes in concentrations of intracellular ions were minimal except for significantly increased tissue magnesium levels at pH 4.5 with aluminum and decreases in all tissue ions in both groups at pH 4.0. Losses of sodium and chloride were very severe at pH 4.0 with and without aluminum, moderately severe at pH 4.5 + aluminum, slight at pH 5.0 + aluminum, and minimal at pH 6.1 + aluminum and pH 4.5 without aluminum. Losses were increased by the presence of organic acids at pH 4.5, being very severe with aluminum and slight without aluminum. However, at pH 5.0 + organic and inorganic aluminum the loss was only minimal. At pH 4.0 there were also significant reductions in tissue potassium and magnesium levels. All of the changes at pH 4.0 were more rapid in the acid only group.

Heavy Metal Free Fulvic Acid Group

Ambient inorganic aluminum levels were similar in the heavy metal free fulvic acid and natural organic acid groups at pH 4.5 with aluminum (Table 1). However, the fulvic acid exposure medium (Table 3) contained approximately 115 μg iron·L^{-1} and 9 μg zinc·L^{-1} compared with 250 μg iron·L^{-1} and 25 μg zinc·L^{-1} in the Dickie 11 organic acid media. Although not strictly heavy metal free, the fulvic acid preparation was sufficiently pure to demonstrate whether or not the toxic effects of the organic acid + aluminum media could be attributed to aluminum.

The fish exposed to heavy metal free fulvic acid + labile inorganic aluminum at pH 4.5 reacted in two different ways. Three fish (nonsurvivors) were active and ventilation rates increased, resulting in high gill aluminum accumulation levels (see Table 4). They died within 3–4 d of exposure. The other three fish (survivors) were calm, had lower ventilation rates and gill aluminum levels, and survived the 6-d exposure period. Their gill aluminum levels were significantly lower than in the natural organic acid + inorganic aluminum group (Fig. 7, row A) and were not associated with coughing, yet there was similar impairment to ionoregulation (Fig. 7, row C). Acidaemia was also evident but total carbonate levels were not reduced. Haemoglobin levels were increased to a lesser extent than in the natural organic acid group, resulting in a significantly lower oxygen capacity (Fig. 7, row B).

TABLE 3. Heavy metal analysis (total, unfiltered) of fulvic acid exposure medium.

Metal	Concentration (mg·L^{-1})
Cadmium	<0.0002
Cobalt	<0.001
Cromium	0.001
Copper	0.003
Nickel	0.004
Lead	<0.003
Zinc	0.009
Iron	0.115
Manganese	0.015

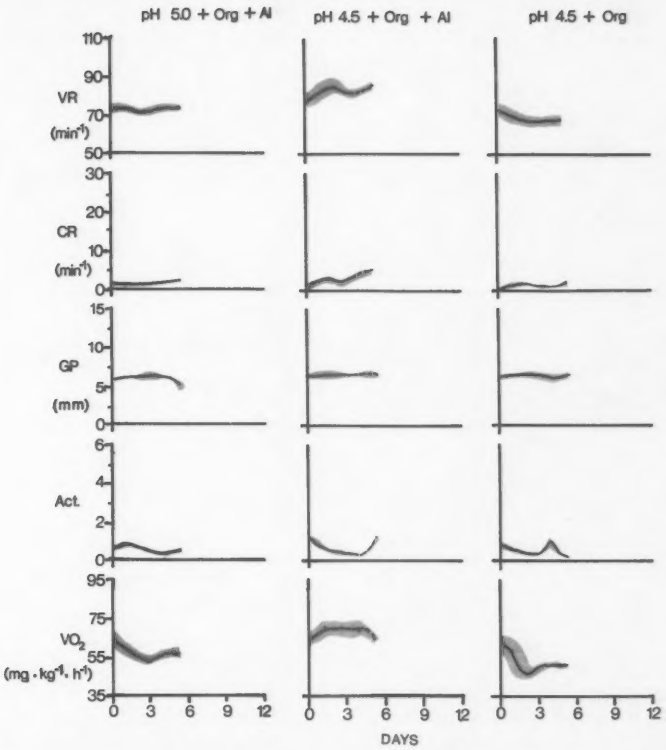


FIG. 3. Ventilation and activity responses throughout the exposure period to acid with (pH 5.0 and 4.5) and without (pH 4.5) 2.8 μM labile inorganic aluminum in the presence of ≈8–10 mg natural organic acids (DOC)·L^{-1} (···, 10% of fish only; ---, 60% of fish only). Org. = organic acid; Al = 2.8 μM labile inorganic aluminum. See Fig. 1 for definition of parameters on y-axis.

Discussion

The groups that experienced a severe initial response to acid and aluminum exposure were also the most severely stressed at the end of the exposure period. However, there was no consistent correlation between the severity of initial responses and the degree of imbalance in either acid–base regulation or ionoregulation. All of those groups became acidaemic but in some cases the acidaemia was due to respiratory acidosis (CO_2 retention), in others to a metabolic acidosis (increased lactic acid, as a result of anaerobic respiration), and in others could not be primarily attributed to either. The presence of organic acids increased the acidaemia but reduced the ventilation response. Increased ventilation rates were sufficient to adequately saturate the arterial blood with oxygen in some groups, but were not able to do so in one of the least acidic groups with aluminum (pH 6.1) or in the most acidic group without aluminum (pH 4.0). Electrolyte levels were severely depleted in the more acidic groups but hardly affected in the near neutral groups. The initial response was therefore indicative of the degree of stress but not the mode of stress.

The response of the fish to the aluminum at the end of the exposure period depended primarily on the pH of the system. Although ambient calcium concentrations varied according to the efficiency of the reverse osmosis units (Table 1), the concentration range used (≈2–5 mg·L^{-1}) did not appear to have a consistent influence on the toxicity of the exposure media. Brown (1983) found that 1.0 mg·L^{-1} was a critical ambient calcium concentration when brown trout were exposed to acid + aluminum. Survival was much greater with ambient calcium concentrations above than below 1.0 mg·L^{-1} at pH 4.5–5.5.

Tissue aluminum levels showed that at the ambient concentrations of labile inorganic aluminum used in these experiments, and typically found in streams and runoff during snowmelt, there was no net uptake of aluminum in the intestine, liver, kidney, or gonads during the 6- to 11-d exposure period. However, aluminum did accumulate on the gills and has been shown by electron microscopy and X-ray diffraction studies to be not only on the surface but also within the cytoplasm of gill epithelial cells (J. H. Youson and C. M. Neville, in prep.).

Effect of Aluminum at Different pH Levels

pH 6.5–5.5 exposures

The gradually developing, mild, ventilatory response to 2.8 μM inorganic aluminum at pH 6.5 and to 1.6 μM inorganic aluminum at pH 6.1 (Fig. 1) shows that the aluminum was gradually detected as an irritant by the fish. The transient nature of the response indicates that for the first few days some aluminum could be cleared from the gills by coughing, and that

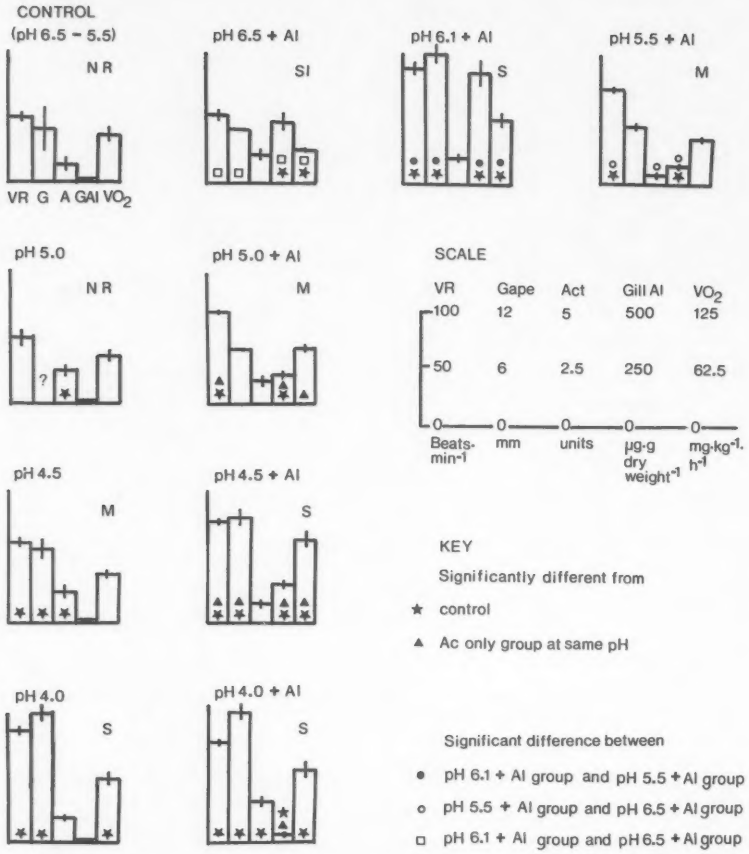


FIG. 4. Ventilation rate (VR), gape (G), activity (A), gill aluminum (GAl), and oxygen consumption rate (VO_2) (mean ± SE) on the last day of the exposure period in the acid and acid + 2.8 µ*M* inorganic aluminum groups, including the significant differences between groups ($P < 0.05$). NR (no response), Sl (slight), M (moderate), and S (severe) refer to the severity of the group ventilation and activity responses to the exposure medium during the first 1–2 h of exposure (see Table 2).

subsequent accommodation to the aluminum was possible. Since the much lower concentration at pH 6.1 produced a similar reaction to that at pH 6.5, it appears that the severity of aluminum toxicity was increased by the slight increase in H^+ concentration.

This is also shown by the very severe reaction at pH 6.1 when the aluminum concentration was the same as that at pH 6.5. After only 24 h of exposure and little activity, the fish could not take up sufficient oxygen despite the tremendous increase in ventilation effort, due to the high concentration of aluminum on the gill surface. Normal body metabolism under resting conditions could not be maintained even with the help of energy supplied by anaerobic respiration (Fig. 5), but electrolyte levels were only slightly affected (Fig. 6). (Electrolyte losses would only become severe when the fish became moribund.) The slight reduction in plasma chloride over approximately 8 d of exposure can therefore be attributed to decreased efficiency of active chloride uptake caused by reduced energy levels, since the blood oxygen saturation was less than half of normal values. Comparison of the probable amount of chloride lost over 8 d with the rate of chloride uptake in resting, adult, freshwater salmonids at neutral pH conditions (Cameron 1976; McDonald et al. 1983) supports this view. At this pH and aluminum concentration, therefore, the direct cause of death was hypoxia.

Even though less aluminum accumulated on the gills at pH 5.5 + 2.8 µ*M* inorganic aluminum than at pH 6.1 and 6.5, the reaction was less severe than at pH 6.1 and greater than at pH 6.5. This was partly due to the shorter exposure period, partly to the higher cough rate which would have cleared much of the aluminum and mucous from the gill epithelial surface, and probably partly to the greater mixing time of the stock aluminum solution and the exposure medium in the recirculation system (0.5–24 h) which could have allowed some of the monomeric inorganic aluminum to polymerize and precipitate. Stress at this pH was again due to increasing difficulty in oxygen uptake at the gill surface rather than to electrolyte loss.

Muniz and Leivestad (1980b) have shown 33.3 µ*M* (900 $\mu g \cdot L^{-1}$) aluminum added to water at pH 6.0 to be nontoxic to brown trout, but they noted that measured water aluminum concentrations did not reflect the amount of aluminum added. Possibly, at

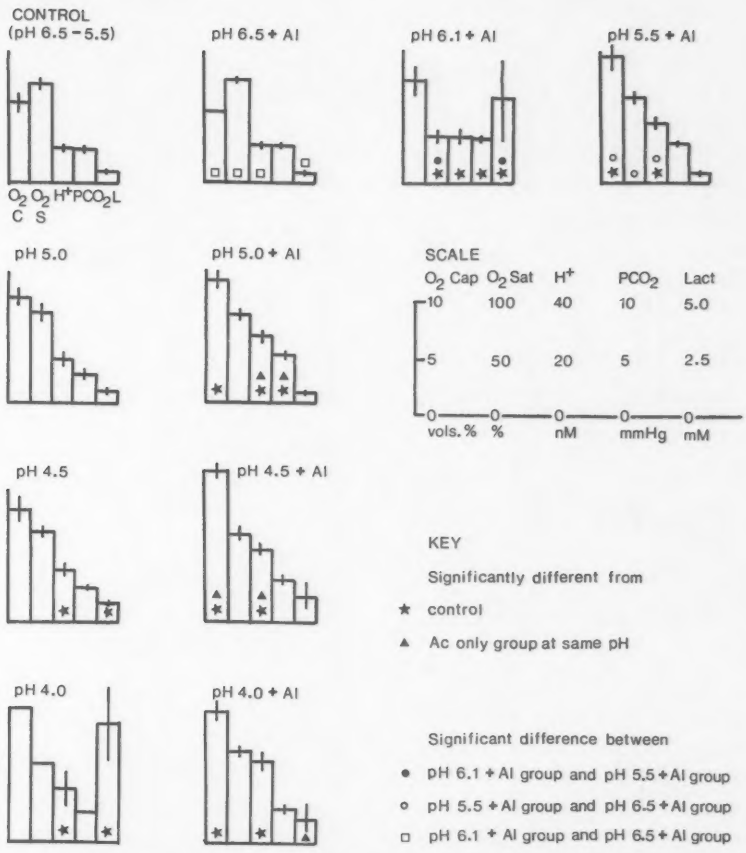

CONTROL
(pH 6.5 – 5.5)
pH 6.5 + Al
pH 6.1 + Al
pH 5.5 + Al
O2 C O2 S H+ PCO2 L
pH 5.0
pH 5.0 + Al
SCALE
O2 Cap O2 Sat H+ PCO2 Lact
10 100 40 10 5.0
5 50 20 5 2.5
0 0 0 0 0
vols.% % nM mmHg mM
pH 4.5
pH 4.5 + Al
KEY
Significantly different from
★ control
▲ Ac only group at same pH
pH 4.0
pH 4.0 + Al
Significant difference between
● pH 6.1 + Al group and pH 5.5 + Al group
○ pH 5.5 + Al group and pH 6.5 + Al group
□ pH 6.1 + Al group and pH 6.5 + Al group

FIG. 5. Arterial blood oxygen capacity (O_2 C), oxygen saturation (O_2 S), hydrogen ion concentration (H^+), carbon dioxide tension (Pco_2), and lactate (L) levels (mean ± SE) on the last day of the exposure period in the acid and acid + 2.8 μ*M* inorganic aluminum groups, including the significant differences between groups ($P < 0.05$).

that concentration, the aluminum formed polymers too large to adhere closely to the gill epithelium.

pH 5.0 exposures

The increased activity and occasional struggling of fish noted in the pH 5.0 exposures with and without aluminum, compared with the control fish, indicated the recognition of an irritant in the water. Without aluminum the irritant caused little other response after 11 d of exposure, but with 2.8 μ*M* inorganic aluminum, oxygen uptake was impaired. However, the 80% arterial blood oxygen saturation maintained by increasing the ventilation rate is still adequate for maintenance of all oxygen-dependent physiological processes unless activity levels are further increased. Hence, the slight loss of electrolytes in this group may be due to slightly increased permeability of the gill epithelium rather than impaired active uptake mechanisms.

The relatively mild response to 2.8 μ*M* aluminum at pH 5.0 is in sharp contrast with the severe response and electrolyte loss noted at higher aluminum concentrations (≈7–17 and 33.3 μ*M*) in rainbow trout and other salmonids (Muniz and Leivestad 1980b; Rosseland 1980; Rosseland and Skogheim 1982).

pH 4.5 exposures

The continued coughing of fish in the acid only group at pH 4.5 indicated that a more harmful irritant was present in the water than at pH 5.0. Under the test conditions the irritant could only have been the greater concentration of hydrogen ions (30 μ*M*). Even so, without aluminum, tissue damage was probably minimal, since there was little change in biochemical parameters apart from a slight loss of plasma chloride. However, at this pH, 2.8 μ*M* inorganic aluminum was sufficient to cause severe tissue damage (J. H. Youson and C. M. Neville, in prep.) with major disturbances in oxygen uptake efficiency and ionoregulation. The sodium and chloride losses were too great to be attributed only to influx of ambient water and/or breakdown of active uptake mechanisms as noted at lower pH values without aluminum (McDonald et al. 1983). The inorganic aluminum must have exacerbated the slight increase in gill permeability apparently caused by the hydrogen ions in the pH 4.5 acid only group.

pH 4.0 exposures

The hydrogen and calcium ion concentrations in the acid only

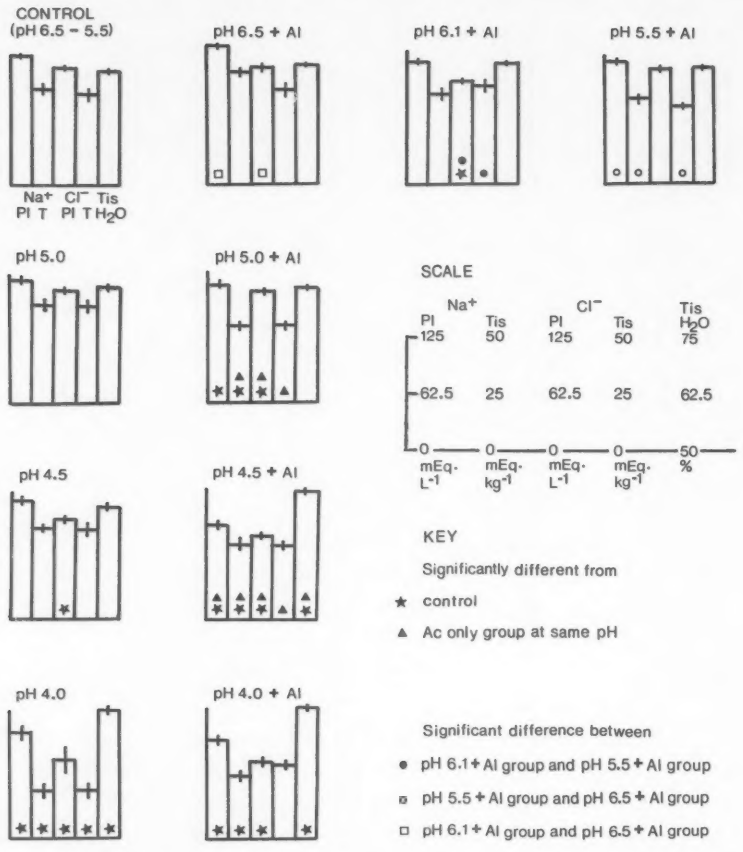

CONTROL
(pH 6.5 – 5.5)
pH 6.5 + Al
pH 6.1 + Al
pH 5.5 + Al
Na+ Cl− Tis
Pl T Pl T H2O
pH 5.0
pH 5.0 + Al
SCALE
Na+
Cl−
Pl Tis Pl Tis Tis H2O
125 50 125 50 75
62.5 25 62.5 25 62.5
0 0 0 0 50
mEq·L-1 mEq·kg-1 mEq·L-1 mEq·kg-1 %
pH 4.5
pH 4.5 + Al
KEY
Significantly different from
★ control
▲ Ac only group at same pH
pH 4.0
pH 4.0 + Al
Significant difference between
● pH 6.1 + Al group and pH 5.5 + Al group
▫ pH 5.5 + Al group and pH 6.5 + Al group
□ pH 6.1 + Al group and pH 6.5 + Al group

FIG. 6. Plasma (Pl) and muscle tissue (T) sodium and chloride concentrations and the muscle tissue water concentration (Tis H_2O) (mean ± SE) on the last day of the exposure period in the acid and acid + 2.8 μM inorganic aluminum groups, including the significant differences between groups ($P < 0.05$)

group produced the most severely toxic conditions of all the test exposures. The reduction in oxygen transport and the amount of sodium and chloride loss within 2–3 d indicate impaired active uptake of these ions as well as an increase in gill epithelial permeability. Again, this agrees with rates of influx and efflux of sodium and chloride reported in rainbow trout at pH 4.0–4.1 by McDonald et al. (1983).

With 2.8 μM inorganic aluminum the toxicity of the acid was reduced rather than increased; therefore, it appears that the low concentration of aluminum accumulated on the gill tissue at this pH afforded some protection to the gill epithelium. However, the protection was only transient. Survival time of the pH 4.0 + aluminum group was similar to the pH 4.5 + aluminum group. Muniz and Leivestad (1980b) have also shown reduction of the acid toxicity to juvenile brown trout at pH 4.0 when inorganic aluminum is present.

Fulvic Acid Exposure

In the fulvic acid + aluminum experiment, the three fish that died had had higher activity rates and subsequent higher ventilation rates and levels of gill aluminum than the surviving fish (Table 3). This emphasizes the extreme depletion of energy levels caused by the acid + aluminum exposure at pH 4.5, and the probable inability of fish to survive heavy energy expenditure in the field.

The surviving fish in the heavy metal free fulvic acid + aluminum and natural organic acid + aluminum groups all developed the typical acidaemia noted in the organic acid exposed fish, and showed similar levels in electrolytes and most of the other biochemical parameters (Fig. 7b). Hence, the response of the natural organic acid + aluminum group during the short-term (6-d) exposure must have been due primarily to the aluminum and not to any heavy metals attached to the organic acid polymers.

Natural Organic Acid Exposure

Reduced activity, moderation of the increase in all ventilation parameters (resulting in lower levels of gill aluminum accumulation), increased acidaemia, and slightly increased ionic imbalance appear to be typical responses of rainbow trout exposed to toxic levels of acid and aluminum when natural organic acids are added (Fig. 3 and 7). Because activity is reduced and oxygen uptake is adequate, the increased acidaemia cannot be solely metabolic or respiratory in origin. Instead, it may be due to

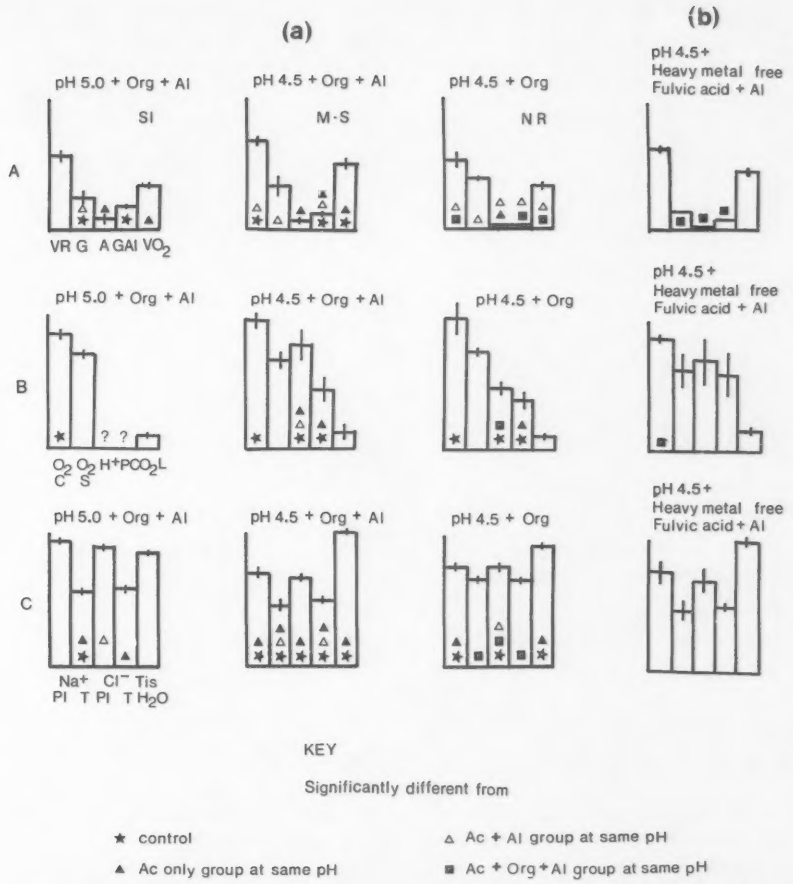


FIG. 7. Ventilation, activity, and gill aluminum (row A), oxygen transport and acid–base (row B), and sodium, chloride, and tissue water (row C) concentrations on the last day of the exposure period in the (a) natural organic acid groups, (b) heavy metal free fulvic acid group showing the significant differences caused by using heavy metal free instead of natural organic acids. See Fig. 4, 5, and 6 for explanation of parameter labels in rows A, B, and C, respectively, and for histogram scales.

TABLE 4. Comparison of activity, ventilation parameters, and gill aluminum concentration between surviving and nonsurviving fish in the fulvic acid + aluminum experiment at pH 4.5 (activity and ventilation parameters, day 2; gill aluminum concentration, day 3 (nonsurvivors) or day 6 (survivors); *significant difference between groups ($P < 0.05$), one-way ANOVA).

Group	Ventilation rate (min^{-1})	Cough rate (min^{-1})	Gape (mm)	Activity (0–5)	O_2 uptake ($mg \cdot kg^{-1} \cdot h^{-1}$)	Gill Al ($\mu g \cdot g$ dry wt^{-1})
Nonsurvivors	97±1.8 (3)	0.7±0.7 (3)	8±1 (3)	2±0 (3)	193±45 (3)	155 (1)[a]
Survivors	75±6.7 (3)*	0.3±0.3 (3)	5±1 (3)	0.7±0.3 (3)*	77±9.7 (3)*	53.3±2.3 (3)*

[a]Gill tissue sample taken immediately after fish died.

increased permeability of the gill epithelium allowing increased passive influx of ambient hydrogen ions or efflux of bicarbonate ions from the fish and/or changes in the rate of active transport of these ions across the cell membrane, causing retention of metabolically produced hydrogen ions. Both situations are consistent with the increased rate of sodium and chloride loss at pH 4.5 (see Fig. 7). However, in electron microscopy studies there appeared to be thickening of cell junctions at the surface of the gill epithelium in the pH 4.5 + organic acid groups (J. H. Youson, pers. comm.). Such thickening could be a response promoted by membrane permeability changes.

Although eventually the organic acids increase the degree of acidaemia, they appear to delay the initial effect of hydrogen and aluminum ions on the gill membrane (Table 3). Both the initial delay and the eventual increase could be due to the buffering properties of organic acids in the ambient water which take effect at pH levels below the pH range of the bicarbonate buffer system. Comparison of responses between the inorganic

TABLE 5. Prediction of detection and avoidance or survival of pH, aluminum, and calcium concentrations in the field similar to those used in the test exposure (calcium ≈ 2–5 mg·L^{-1}).

pH	Labile inorganic aluminum conc. (μg·L^{-1})	DOC (mg·L^{-1})	Detection (within minutes)	Likelihood of avoidance during first 24 h after detection	Likelihood of survival during 6–11 d of exposure (i.e. if avoidance not possible)
6.5	75	—	(+)[a]	+	+
6.1	75	—	+	(±)[b]	−
6.1	45	—	(+)[a]	+	+
5.5	75	—	+	(+)[b]	(±)[c]
5.0	—	—	−	(−)[d]	+
5.0	75	—	+	+	+
5.0	75	8–10	(+)[e]	(−)[e]	+
4.5	—	—	+	+	+
4.5	75	—	+	(±)[b]	−
4.5	75	8–10	+	(±)[b]	−
4.5	—	8–10	−	(−)[d]	+
4.0	—	—	+	−	−
4.0	75	—	+	−	−

[a]Gradual detection after at least 24 h of exposure.
[b]Avoidance possible if refuge areas are found easily, i.e. sustained strong swimming or frequent struggling and swimming would not be possible.
[c]Fish may succumb, depending on degree of activity.
[d]No stimulation for avoidance behavior.
[e]Stimulation likely to be too weak to promote avoidance behavior.

aluminum and organic acid + inorganic aluminum groups at pH 4.5 also shows that the presence of organic aluminum does not ameliorate the inorganic aluminum toxicity at this pH, and may actually increase it, possibly because the organic acids act as a source for aluminum as well as for hydrogen ions.

Hence the changes in response brought about by the presence of organic acids at the concentration used in this study (i.e. with dissolved organic carbon level = 8–10 mg·L^{-1}) may be due to the rate of exposure of the gill membranes to free hydrogen and aluminum ions.

Summary of Physiological Responses to Acid and Aluminum Exposure

The initial 1–2 h of exposure to 2.8 μ*M* inorganic aluminum caused increased ventilation and activity responses ranging from slight to severe depending on the ambient pH value. The severity of physiological response at the end of the 6- to 11-d exposure period was similar to that of the initial response. There was a severe response at pH 6.1, 4.5, and 4.0 and a slight to moderate response at pH 5.0 and 5.5 but little response at pH 6.5. In the severe response groups, 40–90% of the fish died after a few days of exposure. Death was due primarily to hypoxia at pH 6.1, and to electrolyte loss at pH 4.5 and 4.0. In the moderate response groups there was a transition between the two mechanisms of toxicity.

In the presence of 8–10 mg natural organic acids·L^{-1} at pH 5.0 and 4.5 the ventilation and activity responses to the same concentration of inorganic aluminum were subdued but ultimately there was a greater degree of acidaemia. Electrolyte losses were not affected. The severity of final response was unchanged at pH 5.0 but slightly increased at pH 4.5. The total aluminum level was approximately five times the inorganic aluminum level.

Asphyxiation of the fish at pH 6.1 was a result of a purely physical process, i.e. precipitation of monomeric aluminum on the gill surface; therefore, there is no similar response at this pH to the acid alone.

Compared with the physiological response to acid (this study and Wood and McDonald 1982) the presence of 2.8 μ*M* inorganic aluminum increases the toxicity at pH 4.5 by increasing the rate of electrolyte loss, but reduces the toxicity at pH 4.0. At this pH, oxygen transport and electrolyte levels are reduced by exposure to acid alone despite increases in ventilation rate and amplitude but can be partially compensated by the increased ventilation in the presence of inorganic aluminum.

Mechanism of Toxicity

The physiological responses to acid + 2.8 μ*M* aluminum suggest that the mechanism of toxicity varies with pH due to differences in aluminum speciation. However, the mechanism does not appear to be correlated with the ambient aluminum speciation. Neville (1985) proposed that the pH at the gill surface is likely to be less acidic than the ambient pH because in acid conditions fish either take up acid or lose base across the gill membranes. Consequently, aluminum speciation at the gill surface would also be "less acidic" (i.e. the charge on the aluminum would be reduced) than ambient aluminum speciation. The decrease in aluminum acidity would be indirectly proportional to the aluminum concentration, since it would depend on the ratio of hydrogen to aluminum ions. Hence, the aluminum speciation at the gill surface would be the same in low pH, low aluminum conditions as in higher pH, higher aluminum conditions. This hypothesis explains the apparent conflict with studies in the literature (see above) that show higher aluminum concentrations to be more toxic at pH 5.0 than at pH 4.5.

Possible Fish Responses in the Field

From the differences between experimental and control

groups in activity and ventilation parameters observed during the initial 1- to 2-h exposure period, one can make predictions of possible irritant detection. The likelihood of avoidance behavior by fish in the field under the same conditions of pH, aluminum, calcium, and dissolved organic acid concentrations can be made from increases in activity accompanying ventilation changes observed in the experimental groups. The severity of imbalance in homeostatic mechanisms and the nature of ventilation and activity changes at the end of the exposure period indicate the possibility of survival during 6–11 d of exposure in the field if avoidance is not possible, i.e. severe physiological imbalance in the laboratory indicates unlikelihood of survival in the field. Predictions of possible detection and avoidance or survival, assuming increased activity (and therefore use of energy) in the field compared with the "resting" conditions in the laboratory, are shown in Table 5.

Conditions Predicted to be Lethal if Avoidance is Impossible

pH 6.5–5.5 + 2.8 μM labile inorganic aluminum

A situation similar to that at pH 6.1 with 2.8 μ*M* labile inorganic aluminum is likely to have prevailed in lakes where a fish kill occurred in near neutral pH water. If the pH of water with a high dissolved aluminum concentration is increased sufficiently to cause precipitation, forming a fine suspension of aluminum, the aluminum may adhere to the mucous on the fish gill epithelium during ventilation. Unless the fish can clear the gill epithelium by coughing, they die from asphyxiation during prolonged exposure. The results of this series of experiments suggest that the labile inorganic aluminum concentration that would become toxic would be slightly higher at pH 5.5 and 6.5 than at pH 6.1, since solubility is slightly greater (Smith and Hem 1972), and that the toxicity of the fine suspension would diminish within hours as the size of the aluminum polymers in the suspension increased. However, fish kills such as these are rare in natural situations. They have occurred in Sweden during algal blooms and after liming ponds and streams (see below) but have not been reported in North America. The necessary concentration of inorganic aluminum to form a fine precipitate is much higher than normally found in natural waters, especially in water of high organic acid content. Humic acids can complex with possibly 3 (Gamble et al. 1970) to 10 (Oliver et al. 1983) μmol of cations per milligram carbon in this pH range when the carboxyl binding sites are fully deprotonated (Gamble 1972). As approximately half of the sites would be complexed with other metals, particularly iron, it is probable that about 1.5–5 μmol aluminum (40–135 μg) could be complexed per milligram carbon of the humic acid.

Reported fish kills due to increase in pH of acidic aluminum-rich water — Grahn (1980) reported a fish kill in each of two oligotrophic, low organ content, moderately acidic lakes after a period of heavy summer rains followed by an algal bloom. pH and aluminum data were not recorded until 1 wk after the first fish kill, but during the second fish kill, lake pH changed from 5.4 to 6.0, with total aluminum concentrations in inflowing streams of 360–520 $\mu g \cdot L^{-1}$. Dickson (1983) reported fish kills after liming in Swedish ponds and streams. Water in one stream before liming was pH 4.4 with 350 μg total aluminum $\cdot L^{-1}$ and half a day after liming was pH 6.2–6.4 with 300 μg total aluminum $\cdot L^{-1}$. In those conditions (approximately 2°C), therefore, only 50 $\mu g \cdot L^{-1}$ had precipitated out of the water column in approximately 12 h, leaving much of the aluminum still in a fine suspension and therefore still toxic to fish. Rosseland and Skogheim (1984) documented a liming study in Norway and found no fish kills after liming, but the labile aluminum levels were only 50–65 $\mu g \cdot L^{-1}$ at pH 4.6 before liming and 30–40 $\mu g \cdot L^{-1}$ at pH 6.4–6.8 afterwards.

pH 5.0–4.5 + 2.8 μM labile inorganic aluminum

Lethal concentrations of inorganic aluminum at pH 4.5 and sublethal concentrations at pH 5.0 can often occur in small bodies of water during periods of snowmelt when aluminum-rich acidic runoff enters streams and lakes with little buffering capacity. Natural organic acid concentrations are lowest at this time of year, and moreover, the amount of aluminum complexed by humic acids decreases with pH (Gamble 1972). Such conditions can also occur in the fall when heavy rainfalls follow a dry summer period, but toxic concentrations of inorganic aluminum are reached much less frequently than during snowmelt because of the relatively high humic acid levels at this time. Juvenile fish are more likely to inhabit deeper water than the shallow acid-sensitive streams and littoral zones of lakes which are most commonly affected; so in these conditions spawning adults and developing fry are the vulnerable life stages, both of which are even more sensitive than juveniles, since they have very low energy reserves. The spawning adults would certainly be affected by aluminum concentrations that are toxic to juveniles at pH 4.5 and 5.0, whilst the developing fry would probably be most affected by similar aluminum concentrations at a higher pH range (pH 5.0–5.5), since, unlike older life stages with fully developed gills, the pH at the developing gill surface would be very close to that of the ambient water. Spawning adults would probably be able to avoid the toxic conditions if there were nearby refuge areas. Any fish that survived prolonged aluminum exposure during snowmelt could recover in the summer. However, as they would be weaker than unexposed fish, they would be at a disadvantage in competing for food. Acclimation of fish to aluminum during previous periods of exposure is unlikely, since labile inorganic aluminum levels would usually be too low to produce acclimation reactions, though some temporary acclimation may develop in late spring (Schofield et al. 1981).

Reported fish kills due to fall in pH of moderately acidic, calcium-poor water — Leivestad and Muniz (1976) reported a massive kill of brown trout during snowmelt when the pH dropped from 5.2 to 4.65 in the main Tovdal River and to 4.0 in small tributaries. Aluminum levels were not measured at that time. Henriksen et al. (1984) reported that fish kills of Atlantic salmon and sea trout (*Salmo trutta*) occurred each spring since 1981 in a western Norwegian river. In a study of the toxicity and water chemistry during November–December 1983, presmolt (1+) Atlantic salmon kept in tanks fed by the river water died after a series of short periods of increased acidity during rainfall; pH dropped from 5.9 to 5.1 and labile inorganic aluminum increased from 0 to 50 $\mu g \cdot L^{-1}$. This low concentration of aluminum was probably toxic because calcium concentrations were only 0.56–1.15 $mg \cdot L^{-1}$ (i.e. less than half the concentrations used in this study).

The fish kills in the pH range 4.5–5.5 reported from South Norway have occurred in species less sensitive to acidification than rainbow trout (Leivestad et al. 1976). However, the lower species sensitivity is counterbalanced by the extremely low calcium concentration in the lakes of South Norway, typically under 1.0 $mg \cdot L^{-1}$ compared with 2.0–4.0 $mg \cdot L^{-1}$ in North American acid-sensitive lakes. The conditions are therefore

comparable with those predicted to cause fish kills in this study. The lower sensitivity of brook trout, the deeper habitat of lake trout, and the presence of refuge areas explain the lack of reported natural kills of juvenile and adult salmonids due to environmental acidification in North America. However, the sublethal and chronic effects that have been recorded in maturing females (Beamish et al. 1975; Beamish 1976; Schofield 1976) could become acute if there is a reduction in the number and extent of refuge areas that are presently available.

Acknowledgements

I thank Walter Banas, Janet Cairns, John Lee, and Stephen Munger for technical assistance, Dave Sturgis for measurement of aluminum species, the Laboratory Services Branch, Ontario Ministry of the Environment, for water chemical and tissue electrolyte analyses, Drs. D. S. Gamble and B. LaZerte for helpful discussions on the chemistry of natural organic acids and aluminum speciation, Dr. Langford, Concordia University, for supplying and discussing the chemistry of the heavy metal free fulvic acid, and the Ontario Ministry of the Environment for the financial support of this study.

References

ALBERS, C. 1970. Acid–base balance, p. 173–208. *In* W. S. Hoar and D. J. Randal [ed.] Fish physiology. Vol. 4. Academic Press, New York, NY.

BAKER, J. P., AND C. L. SCHOFIELD. 1980. Aluminum toxicity to fish as related to acid precipitation and Adirondack surface water quality, p. 292–293. *In* Proc. Int. Conf. Ecol. Impact Acid Precip. SNSF project, Oslo, Norway.

BEAMISH, R. J. 1974. Loss of fish populations from unexploited remote lakes in Ontario, Canada as a consequence of atmospheric fallout of acid. Water Res. 8: 85–95.

1976. Acidification of Lakes in Canada by acid precipitation and the resulting effects on fishes. Water Air Soil Pollut. 6: 501–514.

BEAMISH, R. J., AND H. H. HARVEY. 1972. Acidification of the La Cloche Mountain Lakes, Ontario, and resulting fish mortalities. J. Fish. Res. Board Can. 29: 1131–1143.

BEAMISH, R. J., W. L., LOCKHART, J. C. VAN LOON, AND H. H. HARVEY. 1975. Longterm acidification of a lake and resulting effects on fishes. Ambio 4: 98–102.

BOOTH, J. H, G. F. JANSZ, AND G. F. HOLETON. 1982. Cl^-, K^+, and acid–base balance in rainbow trout during exposure to, and recovery from, sublethal environmental acidification. Can. J. Zool. 60: 1123–1130.

BROWN, D. J. A. 1983. Effect of calcium and aluminium concentrations on the survival of brown trout (*Salmo trutta*) at low pH. Bull. Environ. Contam. Toxicol. 30: 582–587.

CAMERON, J. N. 1976. Branchial ion uptake in arctic grayling: resting values and effects of acid–base disturbance. J. Exp. Biol. 64: 711–725.

CRONAN, C. S., AND C. L. SCHOFIELD. 1979. Aluminum leaching response to acid precipitation: effects on high-elevation watersheds in the Northeast. Science (Wash., DC) 204: 304–306.

DICKSON, W. 1978. Some effects of the acidification of Swedish lakes. Verh. Int. Ver. Limnol. 20: 851–856.

1983. Liming toxicity of aluminium to fish. Vatten 34: 400–404.

DILLON, P. J., N. YAN, AND H. HARVEY. 1984. Acidic precipitation: effects on aquatic ecosystems. CRC Crit. Rev. Environ. Control 13: 167–194.

DRISCOLL, C. 1984. Procedure for the fractionation of aqueous aluminum in dilute acidic waters. Int. J. Environ. Anal. Chem. 16(4): 267–283.

DRISCOLL, C., J. BAKER, J. BISOGNI, AND C. SCHOFIELD. 1980. Effects of aluminum speciation of fish in dilute, acidified waters. Nature (Lond.) 284: 161–164.

FARMER, G. J., T. R. GOFF, D. ASHFIELD, AND H. S. SAMANT. 1980. Some effects of the acidification of Atlantic salmon rivers in Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 972: 13 p.

FRENETTE, J.-J., AND J. J. DODSON. 1984. Brook trout (*Salvelinus fontinalis*) population structure in acidified Lac Tantaré, Quebec. Can. J. Fish. Aquat. Sci. 41: 865–877.

FROMM, P. O. 1980. A review of some physiological and toxicological responses of freshwater fish to acid stress. Environ. Biol. Fishes 5: 79–93.

GAMBLE, D. S. 1972. Potentiometric titration of fulvic acid: equivalence point calculations and acidic functional groups. Can. J. Chem. 50: 2680–2690.

GAMBLE, D. S., M. SCHNITZER, AND I. HOFFMAN. 1970. Cu^{+2}–fulvic acid chelation equilibrium in 0.1*M* KCl at 25.0°C. Can. J. Chem. 48: 3196–3204.

GRAHN, O. 1980. Fishkills in two moderately acid lakes due to high aluminum concentration, p. 310–312. *In* Proc. Int. Conf. Ecol. Impact Acid Precip. SNSF project, Oslo, Norway.

HAINES, T. A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Trans. Am. Fish. Soc. 110(6): 669–707.

HARVEY, H. H., R. C. PIERCE, P. J. DILLON, J. R. KRAMER, AND D. M. WHELPDALE. 1981. Acidification in the Canadian aquatic environment: scientific criteria for assessing the effects of acidic deposition on aquatic ecosystems. No. 18475. National Research Council of Canada, Ottawa, Ont. 369 p.

HENRIKSEN, A., O. K. SKOGHEIM, AND B. O. ROSSELAND. 1984. Episodic changes in pH and aluminium-speciation kill fish in a Norwegian salmon river. Vatten 40: 255–260.

HOLTZE, K. E. 1983. Effects of pH and ionic strength on aluminum toxicity to early developmental stages of rainbow trout (*Salmo gairdneri* Richardson). MOE Rep. 39 p. (Available from MOE, Dorset Research Centre, Bellwood Acres Road, Dorset, Ont. TOA IEO).

HOWELLS, G. D., D. J. A. BROWN, AND K. SADLER. 1983. Effects of acidity, calcium, and aluminium on fish survival and productivity, a review. J. Sci. Food Agric. 34: 559–570.

JENSEN, K., AND E. SNEKVIK. 1972. Low pH levels wipe out salmon and trout populations in southernmost Norway. Ambio 1: 223–225.

LEIVESTAD, H., G. HENDREY, I. P. MUNIZ, AND E. SNEKVIK. 1976. Effects of acid precipitation on freshwater organisms, p. 87–111. *In* Impact of acid precipitation on forest and freshwater ecosystems in Norway. SNSF project, Oslo, Norway.

LEIVESTAD, H., AND I. P. MUNIZ. 1976. Fish kill at low pH in a Norwegian river. Nature (Lond.) 259: 391–392.

MCDONALD, D. G., R. L. WALKER, AND P. R. H. WILKES. 1983. The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. II. Branchial ionoregulatory mechanisms. J. Exp. Biol. 102: 141–155.

MUNIZ, I. P., AND H. LEIVESTAD. 1980a. Acidification-effects on freshwater fish, p. 84–92. *In* Proc. Int. Conf. Ecol. Impact. Acid Precip. SNSF project, Oslo, Norway.

1980b. Toxic effects of aluminum on the brown trout, *Salmo trutta* L., p. 320–321. *In* Proc. Int. Conf. Ecol. Impact Acid Precip. SNSF project, Oslo, Norway.

NEVILLE, C. M. 1979. Influence of mild hypercapnia on the effects of environmental acidification on rainbow trout. (*Salmo gairdneri*). J. Exp. Biol. 83: 345–349.

1985. Studies on the mechanism of toxicity of acid and aluminum to juvenile trout in a low ion environment (abstract). International Symposium on Acidic Precipitation, Muskoka, Ontario, Canada, Sept. 15–20. Abstr. Water Air Soil Pollut. (In press)

OLIVER, B. G., E. M. THURMAN, AND R. L. MALCOLM. 1983. The contribution of humic substances to the acidity of colored natural waters. Geochim. Cosmochim. Acta 47: 2031–2035.

ROSSELAND, B. O. 1980. Physiological responses to acid water in fish. 2. Effects of acid water on metabolism and gill ventilation in brown trout, *Salmo trutta* L. and brook trout, *Salvelinus fontinalis* Mitchill, p. 348–349. *In* Proc. Int. Conf. Ecol. Impact. Acid Precip. SNSF project, Oslo, Norway.

ROSSELAND, B. O., AND O. K. SKOGHEIM. 1982. Physiological stress and mortality of Atlantic salmon, *Salmo salar* L., in acid water with high levels of aluminum. ICES C.M. 1982/M:29: 15 p.

1984. Attempts to reduce effects of acidification on fishes in Norway by different mitigation techniques. Fisheries 9(1): 10–16.

SCHOFIELD, C. L. 1976. Acid precipitation: effects on fish. Ambio 5: 228–230.

SCHOFIELD, C. L., AND J. R. TROJNAR. 1980. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–366. *In* T. Toribara, M. Miller, and P. Morrow [ed.] Polluted rain. Plenum Press, New York, NY.

SCHOFIELD, C. L., D. A. WEBSTER, C. A. GUTHRIE, AND W. A. FLICK. 1981. Acclimation of brook trout to acidified waters. *In* Management of acidified waters. New York Department of Environmental Conservation and Cornell University, Ithaca, , NY 14853.

SMART, G. 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J. Fish Biol. 8: 471–475.

SMITH, R. W., AND J. D. HEM. 1972. Effect of aging on aluminum hydroxide complexes in dilute aqueous solutions. U.S. Geol. Surv. Water-Supply Pap. 1827-D: 51 p.

SOIVIO, A., K. WESTMAN, AND K. NYHOLM. 1972. Improved method of dorsal aorta catheterization: haematological effects followed for three weeks in rainbow trout (*Salmo gairdneri*). Finn. Fish. Res. 1: 11–21.

TUCKER, V. A. 1967. Method for oxygen content and dissociation curves on microliter blood samples. J. Appl. Physiol. 23(3): 410–414.

WOOD, C. M., AND D. G. MCDONALD. 1982. Physiological mechanisms of acid toxicity to fish, p. 197–226. *In* T. A. Haines [chairman] and R. E. Johnson [ed.] Acid Rain/Fisheries. Proceedings of an International Symposium on Acid Precipitation and Fisheries Impacts in Northeastern North America. Northeast Division of the American Fisheries Society, Bethesda, MD. 357 p.

WRIGHT, R. F., AND E. SNEKVIK. 1978. Acid precipitation: chemistry and fish populations in 700 lakes in southernmost Norway. Verh. Int. Ver. Limnol. 20: 765–775.

Appendix: Comparison of Flowthrough and Recirculation Experiments

As in all of the experiments, the ambient P_{O_2}, P_{CO_2}, and ammonia concentrations were within normal limits even though the latter was higher in the recirculation system (see Results section).

Only one parameter, activity, was significantly different between the flowthrough and recirculation experiments at both of the pH values which were used in both systems (pH 4.5 and 5.0) (see Fig. A.1 for histograms of major parameters). At both pH values the flowthrough groups were slightly restless, while the recirculation groups showed little activity. This was probably due to the longer exposure period of the flowthrough experiments or to a more efficient screening system that was used in the later (recirculation) experiments.

At pH 4.5 + aluminum, in both systems the response was severe. Some fish died and others were killed before the end of the experiment. Those killed showed similar trends in oxygen transport, acid–base balance, ionoregulation, and osmoregulation in samples taken just before killing even though a few individual parameters were slightly but significantly different. These suggested slightly greater stress in the flowthrough group.

In contrast, at pH 5.0 + aluminum the response was mild in both systems. No fish died or were killed before the end of the experiment. The reduced tissue K^+ and Ca^{2+} in the flowthrough groups and higher ventilation with lower plamsa Mg^{2+} in the recirculation groups do not indicate a consistent physiological response but do suggest a slight disturbance in both systems. However, the difference in overall response was greater between the pH 4.5 and 5.0 groups than within each pH group; therefore, the recirculation and flowthrough data for groups exposed to the same conditions were combined.

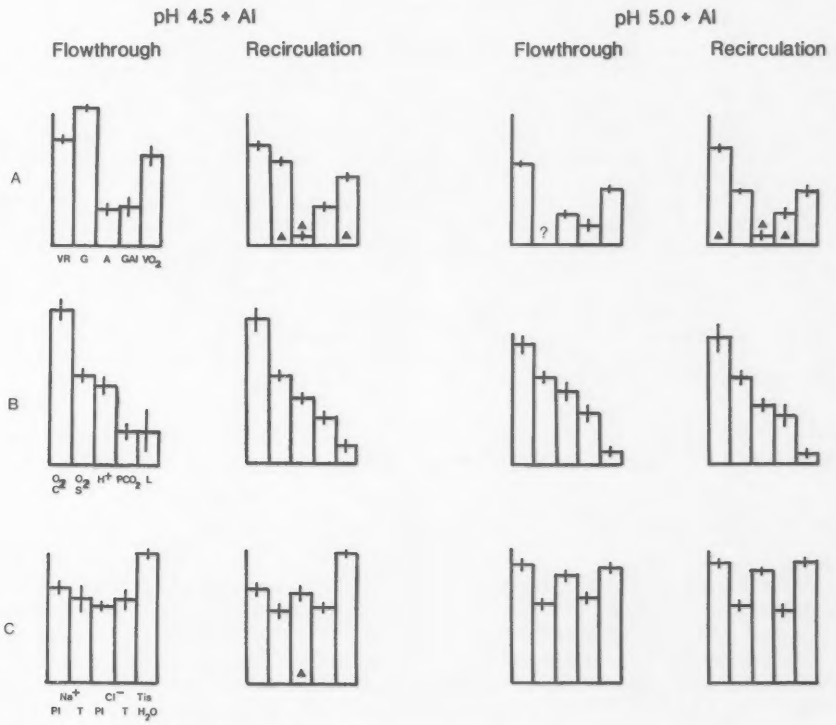


FIG. A.1. Significant differences ($P < 0.05$) in major parameters at the end of the exposure period between the flowthrough and recirculation groups exposed to pH 4.5 + 2.8 μ*M* inorganic aluminum or pH 5.0 + 2.8 μ*M* inorganic aluminum. See Fig. 4, 5, and 6 for explanation of parameter labels in rows A, B, and C, respectively, and for histogram scales.

Variation in Body Morphology Among British Columbia Populations of Coho Salmon, *Oncorhynchus kisutch*

Eric B. Taylor and J. D. McPhail

Institute of Animal Resource Ecology and Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 1W5

Taylor, E. B., and J. D. McPhail. 1985. Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42: 2020–2028.

Ten populations of juvenile coho salmon, *Oncorhynchus kisutch*, from streams tributary to the upper Fraser River, the lower Fraser River, and the Strait of Georgia region were morphologically compared. Juveniles from coastal streams (Fraser River below Hell's Gate and the Strait of Georgia) were more robust (deeper bodies and caudal peduncles, shorter heads, and larger median fins) than interior juveniles. Discriminant function analysis indicated that juvenile coho could be identified as to river of origin with 71% accuracy. Juvenile coho from coastal streams were less successfully classified as to stream of origin; however, juveniles could be successfully identified as either coastal or interior with 93% accuracy. Juvenile coho from north coastal British Columbia, Alaska, and the upper Columbia system also fitted this coastal and interior grouping. This suggests that a coastwide coastal–interior dichotomy in juvenile body form exists. Three populations (one interior and two coastal) were studied in more detail. In these populations the coastal versus interior morphology was consistent over successive years, and was also displayed in individuals reared from eggs in the laboratory. Adult coho salmon also showed some of the coastal–interior morphological differences exhibited by juveniles. We concluded that the morphological differences between coastal and interior coho salmon are at least partially inherited.

Dix populations de saumons cohos juvéniles (*Oncorhynchus kisutch*) de tributaires du haut et du bas Fraser et de la région du détroit de Géorgie ont fait l'objet d'une comparaison morphologique. Les juvéniles des cours d'eau côtiers (Fraser en aval de Hell's Gate et détroit de Géorgie) étaient plus robustes (corps et pédoncule caudal plus massifs, tête plus courte et nageoires médianes plus grandes) que ceux des eaux intérieures. L'analyse par fonction discriminante a permis de déterminer les cours d'eau d'origine des cohos juvéniles avec une exactitude de 71%. Le degré d'exactitude était moindre pour les juvéniles des cours d'eau côtiers, mais il atteignait 93% pour la différenciation des juvéniles des deux types de cours d'eau. Les cohos juvéniles des cours d'eaux côtiers du nord de la Colombie-Britannique, de l'Alaska et de la partie d'amont du réseau du Columbia pouvaient aussi être différenciés en groupes côtier et intérieur. Cela porte à croire à l'existence d'une dichotomie eaux côtières – eaux intérieures de la forme du corps des cohos juvéniles s'étendant à toute la région côtière. Trois populations, une intérieure et deux côtières, ont fait l'objet d'une étude plus approfondie. Chez ces populations, la dichotomie morphologique était constante d'une année à l'autre et apparaissait aussi chez des individus élevés en laboratoire à partir du stade de l'œuf. Les cohos adultes présentaient aussi certaines des différences morphologiques notées chez les juvéniles. Nous en concluons que les différences morphologiques notées entre les saumons cohos des eaux côtières et intérieures sont, du moins en partie, héréditaires.

Received April 29, 1985
Accepted August 12, 1985
(J8220)

Reçu le 29 avril 1985
Accepté le 12 août 1985

The "stock concept" (Simon and Larkin 1972) is widely used in salmonid management (Ricker 1972; McDonald 1981; Fournier et al. 1984). Much of the effort to define populations in *Oncorhynchus* has focused on adult pink (*O. gorbuscha*), chum (*O. keta*), and sockeye (*O. nerka*) salmon (Anas and Murai 1969; Bilton 1971; Beacham and Starr 1982; McGregor 1983; Mulligan et al. 1983). Relatively little effort has gone into defining coho salmon (*O. kisutch*) populations (Ricker 1972); however, in many areas (e.g. the British Columbia south coast and Strait of Georgia), coho, along with chinook salmon (*O. tshawytscha*), form the major component of the commercial troll and sport catch (Argue et al. 1983). Thus, an understanding of the population structure of coho is fundamental to their management.

Coho appear to be less polymorphic at electrophoretically detectable loci than other species of *Oncorhynchus* (Utter et al. 1980; C. F. Wehrhahn and R. Powell, University of British Columbia, Vancouver, B.C., unpubl. data). This does not mean, however, that coho are genetically conservative. Other traits such as behavior and morphology can also provide evidence of interpopulation differentiation (Ihssen et al. 1981a; Riddell et al. 1981). In particular, morphological features (especially those implicated in feeding and locomotion) provide characters in which interpopulation differences can be tested for both their inheritance and their function. In this study we document a suite of morphological differences between coastal and interior coho salmon populations in British Columbia, and attempt to determine if these differences are inherited.

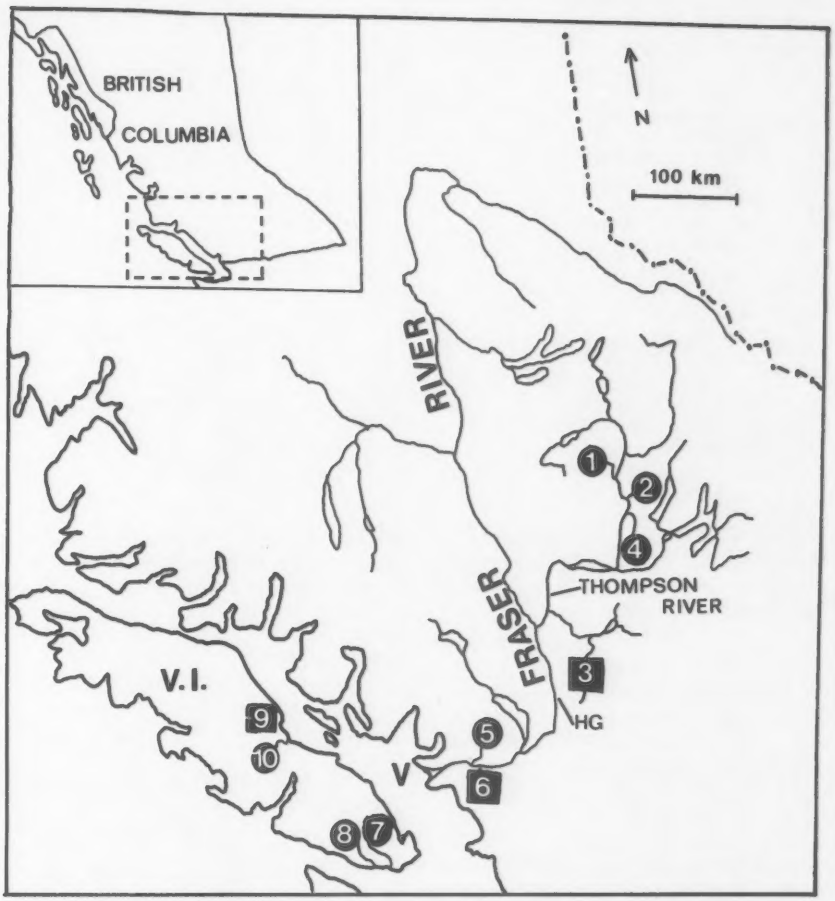


FIG. 1. Geographic locations of study streams. Interior: 1 = Lemieux Cr., 2 = E. Barriere R., 3 = Coldwater R., 4 = Louis Cr.; lower Fraser Valley: 5 = Silverdale Cr., 6 = Wade Cr.; Vancouver Island: 7 = Demamiel Cr., 8 = Kirby Cr., 9 = Morrison Cr., 10 = Rosewall Cr.; V = Vancouver; V.I. = Vancouver Island; HG = Hell's Gate. River and creek lengths are not to scale. Populations indicated by squares are those sampled during successive years and laboratory reared.

Materials and Methods

Juvenile coho were collected with seines, minnow traps, and a Smith-Root type VIII-A electroshocker during 1980, 1982, and 1983. Specimens were preserved in 10% buffered formalin and later transferred to 37.5% isopropyl alcohol. Initially, specimens from 10 populations were collected for morphological analysis, but later we confined collecting to three of these populations: one from the interior (upstream of Hell's Gate on the Fraser River) and two from the coast (Fig. 1).

Body measurements (Table 1) were made to the nearest 0.01 mm using Helios calipers and follow Hubbs and Lagler (1967), except where noted. Included in these measurements are morphological features that other investigators (Webb 1977, 1978; Gatz 1979; Riddell and Leggett 1981) have suggested are important in prolonged and "burst," or fast-start, swimming performance. Median fins were drawn with a Camera Lucida (magnification 12×), and fin areas were then calculated using an APPLE II graphics tablet. Repeated measurements of the same specimens indicated about 3% measurement error for linear measures. The greatest error was in the measurement of caudal peduncle length and fin areas (maximum error about 4%). Unless otherwise indicated, 40 individuals were used to characterize each population.

To determine if the morphological differences apparent in juveniles are retained in adults, mature coho were collected from an interior population (the Coldwater River) and a coastal population (Morrison Creek, Fig. 1). These adults were collected by electroshocking during November 1983. Only fish that had not developed dorsal "humps" and were free of spawning damage were measured. These adults were anesthetized and measured, except for median fin areas, in the same manner as juveniles. Median fin areas in adults were estimated by measuring the approximate triangular dimension of each fin and later calculating the area of the triangle. A comparison of this method with that of drawings made with a Camera Lucida indicated that the "triangulation" method underestimated fin areas by about 10%.

To see if the morphological differences between interior and coastal populations in the Fraser system hold for other areas,

TABLE 1. Morphometric characteristics and their abbreviations used in the text.

Measurement	Symbol	Definition
Standard length	SL	
Body depth	BODYD	
Caudal peduncle depth	PEDD	
Caudal peduncle length	PEDL	
Head length	HL	
Head depth	HD	
Pectoral fin length	PECF	
Pelvic fin length	PELV	Hubbs and Lagler 1967
Dorsal fin area	DFA	
Anal fin area	AFA	
Caudal fin area	CFA	Area bounded by fin margins
Total median fin area	TMFA	Sum of DFA, AFA, and CFA
Snout–anterior insertion PECF	SNAP	
Snout–anterior insertion PELVF	SNAPE	Riddell and Leggett 1981
Mean of BODYD and max. body width	BWBD	
Fineness ratio	FR	Gatz 1979

juveniles from the upper Columbia system (University of Washington Fish Museum) and from creeks in north coastal British Columbia and Alaska (University of British Columbia Fish Museum) were measured and analyzed.

Breeding Experiments

To determine if the morphological differences between wild samples are inherited, individuals from three populations were reared in the laboratory from eggs held under identical conditions. During November 1982, zygotes were collected by mixing the gametes of a small number of males and females at each of the three sites: Morrison Creek (three females × three males), Wade Creek (two females × two males), and the Coldwater River (four females × three males). The eggs were fertilized in the field, transported to the laboratory, and then incubated under controlled conditions (Murray 1980). The incubation apparatus was large enough to accommodate all eggs; thus, all fry experienced the same incubation environment. The temperature control system was set for 4°C and the mean (±SD) temperature was 3.95 ± 0.51°C over the 5-m incubation period.

On emergence, all the fish were removed from the incubators and placed in 92-L rectangular rearing troughs. They were allowed a further 24 h to complete emergence. Wild and laboratory-reared juveniles were maintained in rearing troughs supplied with a constant flow of dechlorinated water. Temperatures during the rearing period fluctuated from 4.0°C in January to 13.5°C in September. For the first 2 wk, fry were fed ad libitum an equal mixture of commercial hatchery food and Tetramin aquarium fish food. Thereafter, fish were fed three times daily on hatchery food. Individual rearing troughs were illuminated by single 40-W cool-white fluorescent lights set to a light–dark cycle that followed natural photoperiod. When the juveniles reached about 50 mm (standard length), 40 individuals from each population were preserved in 10% buffered formalin. They were then transferred to isopropyl alcohol and stored for morphometric analysis.

Statistical Methods

To remove the effects of size, all morphometric data were adjusted to an overall sample mean standard length (Thorpe 1976; Ihssen et al. 1981b; Riddell and Leggett 1981). Logarithmically (base 10) transformed measurements were regressed against the logarithm of standard length in each population and the resultant slopes used to calculate the adjusted data set for each characteristic. The adjustment procedure follows Steel and Torrie (1980). The antilogs of the adjusted data set were used in all subsequent morphological analyses. Because body measurements are intercorrelated and multivariate analysis supports the concept of co-adaptation of body parts (Gould and Johnston 1972), multivariate statistical procedures were used in our analysis.

Multivariate analysis of variance (MANOVA) and stepwise linear discriminant function analysis (BMDP:7M, Dixon 1981) were used to test for differences in body form among populations and to identify the subset of characters that contributed most to discrimination among populations. The Wilks' lambda statistic and its approximate transformation to the F statistic (Dixon 1981) were the criteria used to test for differences between population centroids in the MANOVA. Mahalanobis D^2 was used to summarize morphological distance between population means. All calculations follow formulas given in Dixon (1981). Homogeneity of sample variance–covariance matrices was tested by Box's test (Fox and Gulre 1976). The majority of these latter tests suggested significant differences between variance–covariance matrices ($p < 0.05$). Since our sample sizes were large (i.e. >25) and usually equal, the results of MANOVA are probably not seriously compromised, and subsequent discriminant analysis is justified (Lachenbruch 1975; Pimentel 1979). Confidence limits for group centroids were calculated as outlined in Seal (1964, p. 24).

Spearman rank correlation coefficients were calculated between the means of morphological traits for each population and distance of the natal streams to the sea. Distance from uppermost spawning to the sea was determined from Streamside Fishery Officer Reports (Canadian Department of Fisheries and Oceans, 1090 West Pender St., Vancouver, B.C.) and topographic maps.

Results

Morphometrics of Juveniles from Wild Populations

Coho salmon display a high degree of morphological heterogeneity (Table 2). MANOVA indicated significant differences

TABLE 2. Matrix of Mahalanobis D^2 between centroids of populations of juvenile coho. Also indicated are the mean D^2 for coastal vs. coastal (C–C), interior vs. interior (I–I), and coastal vs. interior (C–I) comparisons.

	Lem	EBar	Cw	Lou	Silv	Wade	Dem	Kirb	Morr
EBar	15.7								
Cw	23.8	27.4							
Lou	23.9	16.9	31.1						
Silv	26.1	36.0	22.5	25.3					
Wade	38.9	38.6	7.3	36.8	15.7				
Dem	22.9	27.9	29.9	25.7	7.5	29.0			
Kirb	21.3	24.1	31.4	23.0	14.4	29.5	6.9		
Morr	46.2	35.2	13.2	31.9	19.0	3.7	29.4	30.2	
Rose	38.1	45.8	22.3	30.7	6.9	10.4	15.0	20.1	10.1

Mean I–I = 23.1, mean C–C = 16.5, mean C–I = 29.2

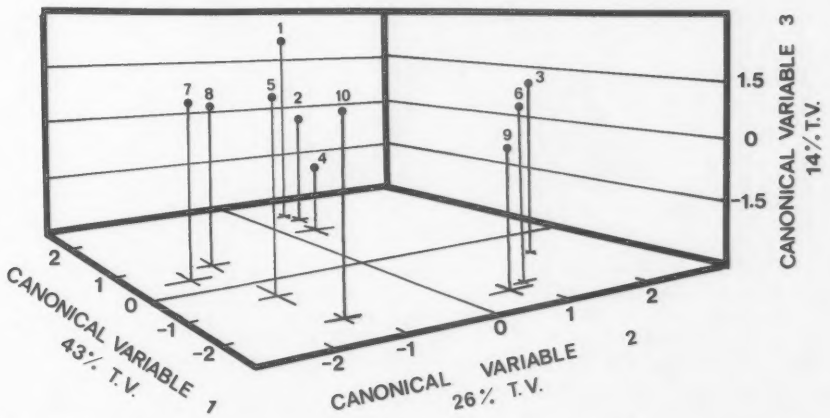


FIG. 2. Population centroids derived from discriminant function analysis of morphological variation among 10 populations of wild juvenile coho salmon. 1 = Lemieux Cr., 2 = E. Barriere Cr., 3 = Coldwater R., 4 = Louis Cr., 5 = Silverdale Cr., 6 = Wade Cr., 7 = Demamiel Cr., 8 = Kirby Cr., 9 = Morrison Cr., 10 = Rosewall Cr. Equations 1–3 are discriminant functions with the most discriminating standardized characters listed; R^2 is the proportion of the total variance accounted for by interpopulation differences:

(1) $\text{df} = 1.77\ \text{BODYD} + 1.73\ \text{DFA} - 4.47\ \text{AFA} - 3.48\ \text{TMFA} - 3.1\ \text{BWBD} + 1.53\ \text{HL} - 11.99, \quad R^2 = 0.76$

(2) $\text{df} = 3.56\ \text{DFA} - 9.76\ \text{AFA} - 2.62\ \text{BODYD} - 1.55\ \text{SNAP} + 2.94\ \text{BWBD} + 1.88\ \text{HL} - 3.29, \quad R^2 = 0.66$

(3) $\text{df} = 0.63\ \text{PEDD} - 0.96\ \text{DFA} - 4.84\ \text{TMFA} + 0.89\ \text{BWBD} + 0.3\ \text{BODYD} - 1.14\ \text{HL} + 10.70, \quad R^2 = 0.51.$

in body form in all pairwise comparisons of population centroids ($F\{12, 408\}$ range: 4.19–62.58). Mahalanobis distance (D^2) values suggested that coastal populations tended to be more similar to other coastal populations than to interior populations. The reverse is also true; however, coastal populations are more similar to one another than interior populations are to one another (Table 2).

A 10-group discriminant function analysis (Fig. 2) using a jack-knifed classification procedure (Lachenbruch and Mickey 1968) correctly classified interior specimens as to river of origin with between 83 and 93% accuracy (mean 88%). In contrast, classification of lower Fraser Valley and Vancouver Island fish was less successful (means 54 and 62%, respectively). Overall, 71% of the individual coho from the 10 populations were correctly classified as to their river of origin. The poorer classification success in coastal populations suggests that there is either greater morphological variation in these populations (and thus more interpopulation overlap) or less differences among means or both. Geographically proximate pairs of coastal populations (e.g. Morrison Creek – Rosewall Creek; Demamiel Creek – Kirby Creek; Wade Creek – Silverdale Creek) were most frequently misclassified (usually with each other), and values for Mahalanobis D^2 between these pairs were usually lower than in general pairwise comparisons (Table 2). This morphological similarity of geographically nearby populations is also reflected in the positive correlation ($r = 0.46$, 43 df, $p < 0.01$) between geographic distance and the D^2 between populations along the first discriminant axis.

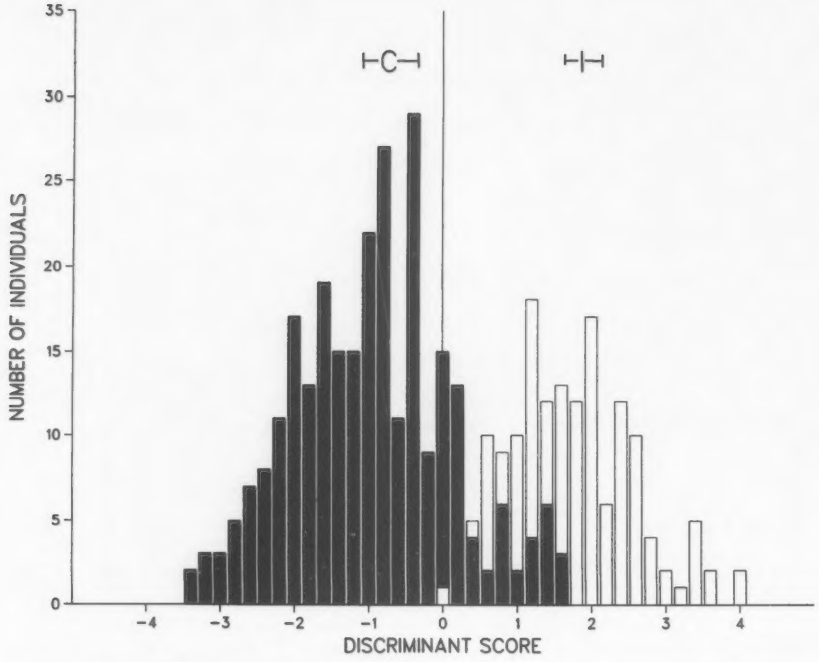


FIG. 3.. Histogram of discriminant scores derived from discriminant analysis of morphological variation between coastal (solid bars, $N = 235$) and interior (open bars, $N = 155$) juvenile coho salmon. C = coastal and I = interior group means (±95% confidence intervals). Equation 4 is a discriminant function with the standardized characters contributing most to the separation listed; R^2 is the proportion of the total variance accounted for by differences between coastal and interior juvenile coho:

(4) $\text{df} = 1.2\,\text{HL} - 8.3\,\text{AFA} - 0.85\,\text{BODYD} - 0.74\,\text{SNAP} + 0.20\,\text{SNAPE} - 1.08\,\text{PEDD} - 6.88, \quad R^2 = 0.63.$

Juvenile coho from the lower Fraser Valley resemble Vancouver Island juveniles more closely ($D^2 = 3.6$) than they resemble upper Fraser River (interior) juveniles ($D^2 = 8.1$). The greatest distinction was between interior and Vancouver Island juveniles ($D^2 = 9.2$). This implies that there are no consistent morphological differences between Vancouver Island and lower Fraser coho juveniles, but that there are consistent differences between these two groups and upper Fraser coho. Thus, we pooled the populations into coastal (Vancouver Island and lower Fraser Valley populations) and interior (upper Fraser) groups and explored the characters that best discriminated between coastal and interior coho. All univariate comparisons were significant (analysis of variance, Table 3). Coastal and interior group centroids were significantly different ($F\{7, 393\} = 94.5$, $p < 0.001$), and the results of a two-group discriminant function analysis suggest a clear separation of coastal and interior coho on the basis of body form (Fig. 3). The variables with the six highest standardized contributions to the discriminant model were AFA, PEDD, HL, BODYD, SNAP, and SNAPE (see Table 1 for definitions). Coastal juveniles were consistently associated with high TMFA, BODYD, and PEDD relative to interior coho. Overall, juveniles could be classified as either coastal or interior in origin with 93% accuracy. Most misclassification involved coastal individuals misclassified as interior (11%), and only three interior juveniles (<2%) were misclassified as coastal fish. The coastal fish also had a greater ($p < 0.05$) generalized variance (a composite of variation for all characters — see also coefficients of variation, Table 3) than interior fish. All median fin areas, BODYD, and PEDD were negatively correlated with the distance from the upper limit of spawning to the sea (Table 4). Again, these results indicate that

TABLE 3. Adjusted (to 48 mm standard length) body measurements (geometric means and CV) of pooled coastal and interior juvenile coho salmon.

Measurement	Coastal ($N = 235$)	Interior ($N = 155$)	$F\{1, 389\}$	p
BODYD (mm)	12.38 (0.057)	11.35 (0.045)	149.2	<0.001
PEDD (mm)	4.99 (0.064)	4.72 (0.048)	93.2	<0.001
PEDL (mm)	14.25 (0.034)	13.91 (0.031)	53.7	<0.001
DFA (mm^2)	34.96 (0.184)	29.72 (0.201)	75.6	<0.001
AFA (mm^2)	31.06 (0.171)	25.42 (0.208)	148.8	<0.001
CFA (mm^2)	84.53 (0.168)	76.63 (0.155)	33.1	<0.001
TMFA (mm^2)	151.65 (0.156)	132.01 (0.146)	75.3	<0.001
PECF (mm)	8.46 (0.063)	8.59 (0.051)	7.1	<0.01
PELVF (mm)	6.70 (0.061)	6.81 (0.051)	8.1	<0.005
SNAP (mm)	12.48 (0.042)	12.35 (0.041)	5.7	<0.025
SNAPE (mm)	25.87 (0.035)	26.15 (0.024)	11.3	<0.001
BWBD (mm)	9.36 (0.064)	8.91 (0.055)	78.9	<0.001
FR	5.12 (0.057)	5.36 (0.050)	67.3	<0.001
HL (mm)	13.91 (0.041)	14.25 (0.036)	35.7	<0.001
HD (mm)	9.08 (0.035)	9.27 (0.049)	20.9	<0.001

TABLE 4. Spearman rank correlation coefficients (df = 8) of body measurements of juvenile coho with the distance of their natal rivers to the sea.

Measurement	r	p
BODYD	−0.83	<0.01
DFA	−0.71	<0.05
AFA	−0.85	<0.01
TMFA	−0.72	<0.01
FR	−0.71	<0.05

TABLE 5. Results of classification procedure on juvenile coho salmon from populations excluded from use in the calculation of the "coastal–interior" discriminant function. Coastal–interior midpoint is 0.38 (Fig. 3). An asterisk indicates a classification error.

Location	Discriminant score	Classification
Coastal		
Unnamed (Q.C.I.)	−0.85	Coastal
	0.35	Coastal
	−0.98	Coastal
	−0.55	Coastal
	0.37	Coastal
Unnamed (Juskatla Inlet)	−2.07	Coastal
	−0.74	Coastal
Martin R. (Ocean Falls)	0.42	Interior*
Agattu Is. (Alaska)	0.36	Coastal
Gold Cr. (Q.C.I.)	2.72	Interior*
Gold Cr. (Q.C.I.)	−0.75	Coastal
Bear Lake (Van. Is.)	3.06	Interior*
Gillies Bay Cr. (Texada Is.)	0.09	Coastal
Interior		
Salmon R. (Columbia R.)	3.70	Interior
War Cr. (Columbia R.)	1.71	Interior
	3.34	Interior
	1.76	Interior
	0.99	Interior
	1.44	Interior
	0.37	Coastal*

interior coho tend to have a less robust body form than coastal coho.

To test the power of the coastal–interior discriminant function, juvenile coho from the upper Columbia system, north coastal British Columbia, the Queen Charlotte Islands, and Alaska (Table 5) were measured and then classified using the "coastal–interior" discriminant function (Fig. 3, equation 4). Ten of 13 coastal individuals were correctly classified as coastal (77%) and 6 of 7 upper Columbia juveniles were correctly classified as interior (86%). This suggests that the "coastal–interior" stock separation found in the Fraser River – Strait of Georgia area is part of a coastwide pattern.

Between-Year Morphological Variation

There were significant differences in body form within populations over the two consecutive years in which wild juveniles were collected; however, the heterogeneity between populations was usually greater than the heterogeneity between years within populations. In only one case was the D^2 greater for an intrapopulation comparison (3 comparisons, range 12.3–29.6) than an interpopulation comparison (12 comparisons, range 28.2–80.9).

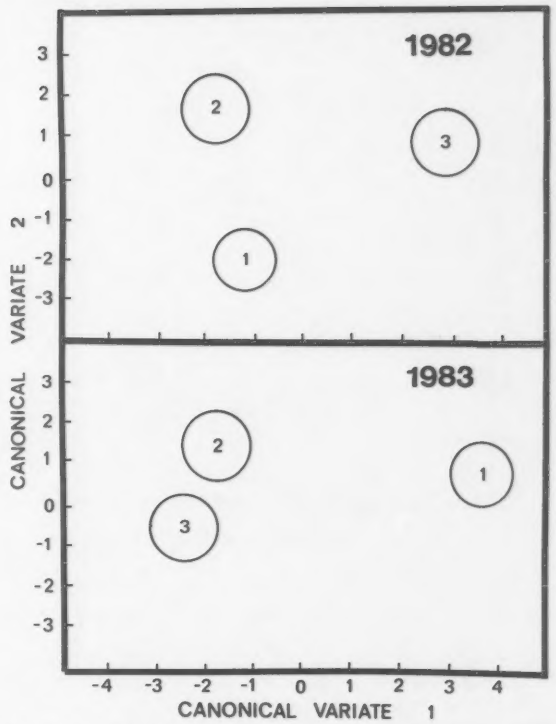


FIG. 4. Population centroids (±95% confidence circles) derived from discriminant function analysis of morphological variation among wild juvenile coho salmon from three populations sampled in 1982 and 1983: 1 = Coldwater R., 2 = Morrison Cr., 3 = Wade Cr. Discriminant functions with the standardized characters contributing most to the separation among populations listed are also given; R^2 is the proportion of the total variance accounted for by interpopulation differences:

1982:

(5) $\mathrm{df} = 9.44\ \mathrm{DFA} + 2.14\ \mathrm{TMFA} - 1.65\ \mathrm{PEDD} - 2.09\ \mathrm{PELVF} - 1.07\ \mathrm{HL} + 32.97, \quad R^2 = 0.80$

(6) $\mathrm{df} = 1.23\ \mathrm{HL} + 0.95\ \mathrm{BWBD} - 6.79\ \mathrm{DFA} - 2.33\ \mathrm{TMFA} + 1.33\ \mathrm{PEDD} - 15.00, \quad R^2 = 0.67.$

1983:

(7) $\mathrm{df} = 8.9\ \mathrm{FR} + 2.45\ \mathrm{BWBD} + 1.37\ \mathrm{HL} - 0.92\ \mathrm{BODYD} + 0.96\ \mathrm{HD} + 0.15\ \mathrm{PEDL} - 92.27, \quad R^2 = 0.86$

(8) $\mathrm{df} = 2.97\ \mathrm{PEDL} - 0.98\ \mathrm{BODYD} - 4.87\ \mathrm{BWBD} - 11.5\ \mathrm{FR} - 0.04\ \mathrm{HL} - 1.61\ \mathrm{HD} + 115.54, \quad R^2 = 0.35.$

Significant interpopulation differences in overall body shape were observed in both the 1982 and 1983 wild samples (MANOVA, minimum $F\{9, 110\} = 29.8$ and $F\{6, 112\} = 17.1$, respectively). In both 1982 and 1983, HL and features of body robustness were the dominant morphological features differentiating the three populations (Fig. 4). Jack-knifed classification correctly identified specimens with 93% accuracy (range 90–98%) in 1982 and with 87% accuracy (range 80–98%) in 1983. Some shifting of centroids between years was evident and this may be due to year-to-year environmental modification of overall morphology (Riddell and Leggett 1981).

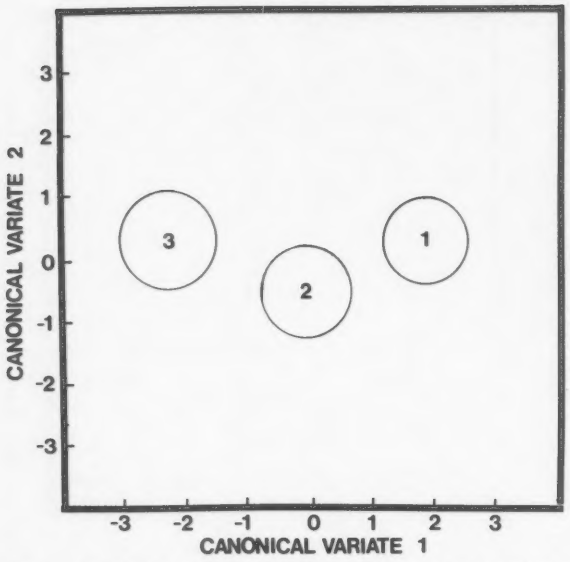


FIG. 5. Population centroids (±95% confidence circles) derived from discriminant function analysis of morphological variation among laboratory-reared coho salmon juveniles from the three study populations: 1 = Coldwater R., 2 = Morrison Cr., 3 = Wade Cr. Equations 9 and 10 are discriminant functions with the standardized characters contributing most to the separation among populations listed; R^2 is the proportion of the total variance accounted for by interpopulation differences:

(9) $\text{df} = 1.85\ \text{PEDL} - 1.56\ \text{BODYD} - 13.23\ \text{DFA} - 0.38\ \text{SNAPE} + 0.81\ \text{HD} + 12.10, \quad R^2 = 0.75$

(10) $\text{df} = 2.92\ \text{HD} - 0.63\ \text{BODYD} - 2.01\ \text{PEDL} + 2.11\ \text{DFA} - 1.04\ \text{SNAPE} + 26.52, \quad R^2 = 0.20.$

Although populations maintained their distinctive body form between years, certain characters (e.g. BODYD, PEDD, BWBD) always contributed more to the discrimination among the three populations than other characters (e.g. fin areas and lengths). Perhaps this difference in constancy of characters over time reflects a greater environmental influence on the expression of the most variable traits.

Laboratory-Reared Populations

The significant differences among populations in body form in the wild samples were maintained in the laboratory (MANOVA, minimum $F\{5, 113\} = 18.5$, $p < 0.001$). Discriminant function analysis (Fig. 5) and jack-knifed classification correctly identified laboratory-reared individuals as to their population of origin with 86% accuracy. A single discriminant function explained 92% of the total variation in morphology in the laboratory-reared populations. The characters contributing most to the discrimination were DFA, PEDL, BODYD, HD, and SNAPE. When individual characters were ranked relative to their magnitude between populations, the character ranking in laboratory samples was correlated with the ranking in the 1983 wild samples ($r = 0.46$, $p < 0.05$, df = 43) but not in the 1982 wild samples. The characters that consistently ranked the same among the three populations in laboratory and wild samples were BODYD, PEDD, HD, FR, and BWBD.

In all wild and laboratory comparisons, Coldwater River (interior) fish had less robust bodies than either of the coastal populations. In addition, Coldwater fish had smaller fin areas in both the 1983 wild and laboratory samples than either of the coastal populations. The characters that "best" differentiated the wild samples also contributed most to differentiating the laboratory-reared populations (i.e. BODYD, HD, median fin areas).

TABLE 6. Adjusted (to 46.5 cm standard length) body measurements (geometric mean ± SD) of adult coho salmon from Morrison Creek and the Coldwater River.

Measurement	Coldwater River ($N = 10$)	Morrison Creek ($N = 11$)	$F\{1, 20\}$	p
BODYD (cm)	10.0 (0.45)	11.1 (0.59)	13.1	<0.005
PEDD (cm)	3.9 (0.22)	4.3 (0.19)	6.7	<0.05
DFA (cm^2)	15.7 (2.05)	20.2 (2.95)	8.7	<0.01
AFA (cm^2)	16.7 (1.84)	20.4 (2.84)	7.9	<0.05
CFA (cm^2)	29.4 (2.69)	38.6 (3.36)	33.8	<0.001
TMFA (cm^2)	61.8 (4.72)	79.2 (7.12)	26.9	<0.001
BWBD (cm)	7.7 (0.50)	8.5 (0.44)	11.5	<0.005
FR	6.2 (0.23)	5.5 (0.28)	25.6	<0.001
HD (cm)	8.5 (0.51)	8.6 (0.61)	0.1	>0.05

Adult Morphometrics

Despite the small number of adults analyzed, the interpopulation trends evident in juvenile samples were also present in adults. Adults from the interior (Coldwater River) had significantly shallower, less robust bodies and smaller median fin areas, and were also less variable in these characters, than Morrison Creek adults (Table 6). Head length was sexually dimorphic in Coldwater River adults but not in Morrison Creek adults. We omitted head length from the interpopulation comparisons.

Discussion

Over the geographic range covered in this study, juvenile coho salmon can be placed into two major morphological "forms": (1) a "coastal" form, with large median fins and deep, robust bodies, and (2) an "interior" form, with small median fins and more streamlined bodies. Geographic populations groups have been suggested for other salmonids (Bilton 1971; Utter et al. 1980; Hjort and Schreck 1982; McGregor 1983; McBride and Marshall 1983) and in other groups of fish (McAllister and Lindsey 1959). Such groups often consist of "coastal" and "interior" or "inland" forms. For example, Utter and Allendorf (1977) suggested that over its native range, *Salmo gairdneri* consists of two major groups: a "coastal" group that extends from Kodiak Island, Alaska, south to the Mad River of northern California and an "inland" group that ranges east of the Cascade Mountains in the Fraser and Columbia River systems. Several other studies also have proposed coastal–interior population groupings for *S. gairdneri* (Gold 1977; Behnke 1981; Parkinson 1984). McBride and Marshall (1983) and Wilcock and McBride (1983) suggested a coastal–interior division for Yukon River chinook salmon.

The high level of correct classification, based on the discriminant function derived in this study (Fig. 3, equation 4), of juvenile coho from north coastal British Columbia, Alaska, and the upper Columbia River system as either coastal or interior suggests that this coastal–interior distinction is geographically widespread in coho. The separation of south coastal and interior British Columbia coho salmon on the basis of morphology is

also supported by clear differences between these groups in smolting behaviour (G. J. Birch, Department of Zoology, University of British Columbia, Vancouver, B.C., pers. comm.) and swimming performance (Taylor and McPhail 1985).

Phenotypic differences among populations can be due to both genetic and environmental differences. In an electrophoretic survey of west coast salmonids, Utter et al. (1980) suggested that coho may have evolved a genome "possessing little genetic variation, but a highly adaptable phenotype." Our data, however, suggest that morphological differences between coho populations are, at least in part, genetically determined. In particular, the morphological distinctness of populations, over successive years and when reared under the same laboratory conditions, argues strongly that their morphological "character" has some genetic basis. Similar genetically influenced differences in morphology between populations are well known in salmonids (Ricker 1972; Riddell et al. 1981) and nonsalmonids (Barlow 1961; McPhail 1984).

The distinctive fusiform morphology of interior coho may represent an adaptation to prolonged swimming. Interior populations of anadromous salmonids, both as smolts and adults, must cope with long and energetically demanding migrations (Idler and Clemens 1959; Gilhousen 1980). Thus, selection should favour characteristics that minimize energy expenditures. Indeed, interior coho exhibit superior prolonged swimming performance relative to coastal coho (Taylor and McPhail 1985) and thus their more fusiform body shape may contribute to an energetically efficient migration. Several other studies involving salmonids have also noted the correlation between a fusiform body and either prolonged swimming performance or migration distance (Evropeitseva 1962; Fessler and Wagner 1969; Vanstone and Markert 1968; Yevsin 1976, 1977; Thomas and Dohahoo 1977).

In contrast with interior populations, the juveniles of coastal coho have more robust bodies. Further, in coastal populations, there is more morphological variation within sites, and thus greater morphological variation among sites (Tables 2 and 3). This suggests that straying, and thus gene flow, may be greater among coastal populations than among interior populations. Straying would erode, or at least slow, the process of deme formation among coastal populations and thus account for their high (relative to interior populations) level of morphological heterogeneity. Alternatively, the selective pressures acting on body form may not be as strong in coastal populations as in interior populations.

Acknowledgments

We thank G. Birch, S. Cox-Rogers, and P. Lavin for assistance during the later field collections. Clyde Murray made the initial synoptic collections of juvenile coho in the Strait of Georgia region and Fraser system. Without these extensive collections, the differences between coastal and interior coho would not have been apparent. P. A. Larkin and P. Lavin made constructive criticisms on the manuscript. Funding was supplied by a Natural Sciences and Engineering Research Council of Canada (NSERC) strategic grant (67-0976) awarded, in part, to J. D. McPhail.

References

ANAS, R. E., AND S. MURAI. 1969. Use of scale characters and a discriminant function for classifying sockeye salmon (*Oncorhynchus nerka*) by contingent of origin. Int. North Pac. Fish. Comm. Bull. 26: 157–192.

ARGUE, A. W., R. HILBORN, R. M. PETERMAN, M. J. STALEY, AND C. J. WALTERS. 1983. Strait of Georgia chinook and coho fishery. Can. Bull. Fish. Aquat. Sci. 211: 91 p.

BARLOW, G. 1961. The causes and significance of morphological variation in fishes. Syst. Zool. 10: 105–117.

BEACHAM, T. D., AND P. J. STARR. 1982. Population biology of chum salmon, *Oncorhynchus keta*, from the Fraser River, British Columbia. Fish. Bull. 80: 813–825.

BEHNKE, R. J. 1981. Systematics and zoogeographical interpretation of Great Basin trouts, p. 95–124. *In* R. J. Naiman and D. L. Soltz [ed.] Fishes in North American deserts. Wiley, New York, NY. 552 p.

BILTON, H. T. 1971. Identification of major British Columbia and Alaska runs of even-year and odd-year pink salmon from scale characters. J. Fish. Res. Board Can. 29: 295–301.

DIXON, W. J. [ED.] 1981. BMDP statistical software. University of California at Los Angeles Press, Los Angeles, CA 880 p.

EVROPEITSEVA, N. V. 1962. Comparative analysis of the desmoltification process among the young of different ecological forms of Atlantic salmon. Uch. Zap. Leningr. Gos. Univ. 311: 46–73. (Transl. from Russian by Fish Res. Board Can. Transl. Ser. 431)

FESSLER, J. L., AND H. F. WAGNER. 1969. Some morphological and biochemical changes in steelhead trout during the parr–smolt transformation. J. Fish. Res. Board Can. 26: 2823–2841.

FOURNIER, D. A., T. D. BEACHAM, B. E. RIDDELL, AND C. A. BUSACK. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41: 400–408.

FOX, D. L., AND K. E. GULRE. 1976. Documentation for MIDAS. 3rd ed. Statistical Research Laboratory, University of Michigan, Ann Arbor, MI. 203 p.

GATZ, A. J. 1979. Ecology and morphology of freshwater stream fishes. Tulane Stud. Bot. Zool. 21: 91–123.

GILHOUSEN, P. 1980. Energy sources and expenditures in Fraser River sockeye salmon during their spawning migration. Int. Pac. Salmon Fish. Comm. Bull. 22: 51 p.

GOLD, J. R. 1977. Systematics of western North American trout (*Salmo*), with notes on the redband trout of Sheepheaven Creek, California. Can. J. Zool. 55: 1858–1873.

GOULD, S. J., AND J. T. JOHNSTON. 1972. Geographic variation. Annu. Rev. Ecol. Syst. 3: 457–498.

HJORT, R. C., AND B. SCHRECK. 1982. Phenotypic differences among wild and hatchery stocks of coho salmon in Oregon, Washington and California. Fish. Bull. 80: 105–119.

HUBBS, C. L., AND K. F. LAGLER. 1967. Fishes of the Great Lakes region. University of Michigan Press, Ann Arbor, MI. 213 p.

IDLER, D. R., AND W. A. CLEMENS. 1959. The energy expenditure of Fraser River sockeye salmon during migration, spawning and death. Int. Pac. Salmon Fish. Comm. Prog. Rep. 6: 80 p.

IHSSEN, P. E., H. E. BOOKE, J. M. CASSELMAN, J. M. MCGLADE, N. R. PAYNE, AND F. M. UTTER. 1981a. Stock identification: materials and methods. Can. J. Fish. Aquat. Sci. 38: 1838–1855.

IHSSEN, P. E., D. O. EVANS, W. J. CHRISTIE, J. A. RECKAHN, AND R. L. DESJARDINE. 1981b. Life history, morphology, and electrophoretic characteristics of five allopatric stocks of lake whitefish (*Coregonus clupeaformis*) in the Great Lakes region. Can. J. Fish. Aquat. Sci. 38: 1790–1807.

LACHENBRUCH, P. 1975. Discriminant analysis. Hafner Press, New York, NY. 128 p.

LACHENBRUCH, P., AND R. M. MICKEY. 1968. Estimation of error rates in discriminant analysis. Technometrics 10: 1–11.

MCALLISTER, D. E., AND C. C. LINDSEY. 1959. Systematics of the freshwater sculpins (*Cottus*) of British Columbia. Natl. Mus. Can. Bull. Contrib. Zool. 172: 66–89.

MCBRIDE, D. N., AND S. L. MARSHALL. 1983. Feasibility of scale pattern analysis to identify the origins of chinook salmon (*Oncorhynchus tshawytscha* Walbaum) in the lower Yukon River commercial gillnet fishery, 1980–1981. Alaska Dep. Fish Game Inf. Leafl. No. 208: 64 p.

MCDONALD, J. 1981. The stock concept and its application to British Columbia salmon fisheries. Can. J. Fish. Aquat. Sci. 38: 1657–1664.

MCGREGOR, A. J. 1983. A biochemical genetic analysis of pink salmon (*Oncorhynchus gorbuscha*) from selected streams of northern Southeast Alaska. Alaska Dep. Fish Game Inf. Leafl. No. 213: 70 p.

MCPHAIL, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Can. J. Zool. 62: 1402–1408.

MULLIGAN, T. J., L. LAPI, R. KIESER, S. B. YAMADA, AND D. L. DUEWER. 1983. Salmon stock identification based upon elemental composition of vertebrae. Can. J. Fish. Aquat. Sci. 40: 215–229.

MURRAY, C. B. 1980. Some effects of temperature on zygote and alevin survival, rate of development and size at emergence of Pacific salmon and rainbow trout. M.Sc. thesis, University of British Columbia, Vancouver, B.C. 156 p.

PARKINSON, E. A. 1984. Genetic variation among populations of steelhead trout (*Salmo gairdneri*) in British Columbia. Can. J. Fish. Aquat. Sci. 41: 1412–1420.

PIMENTEL, R. A. 1979. Morphometrics: the multivariate analysis of biological data. Kendall/Hunt Publ. Co., Dubuque, IA. 276 p.

RICKER, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations, p. 19–160. *In* R. C. Simon and P. A. Larkin [ed.] The stock concept in Pacific salmon. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C. 231 p.

RIDDELL, B. E., AND W. C. LEGGETT. 1981. Evidence for an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 38: 308–320.

RIDDELL, B. E., W. C. LEGGETT, AND R. L. SAUNDERS. 1981. Evidence of adaptive polygenic variation between two populations of Atlantic salmon (*Salmo salar*) native to tributaries of the S.W. Miramichi River, N.B. Can. J. Fish. Aquat. Sci. 38: 321–333.

SEAL, H. L. 1964. Multivariate statistical analysis for biologists. Methuen and Co., London. 269 p.

SIMON, R. C., AND P. A. LARKIN [ED.] 1972. The stock concept in Pacific salmon. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C. 231 p.

STEEL, R. G. D., AND J. H. TORRIE. 1980. Principals and procedures of statistics. 2nd ed. McGraw Hill Book Co., New York, NY. 633 p.

TAYLOR, E. B., AND J. D. MCPHAIL. 1985. Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42: 2029–2033.

THOMAS, A. E., AND M. J. DOHAHOO. 1977. Differences in swimming performance among strains of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 304–306.

THORPE, R. S. 1976. Biometric analysis of geographic variation and racial affinities. Biol. Rev. 51: 407–452.

UTTER, F. M., AND F. W. ALLENDORFF. 1977. Determination of the breeding structure of steelhead populations through gene frequency analysis, p. 10–15. *In* T. J. Hassler and R. R. VanKirk [ed.] Genetic implications of steelhead management. Calif. Coop. Fish. Res. Unit Spec. Rep. 77-1, Arcata, CA.

UTTER, F. M., D. CAMPTON, S. GRANT, G. MILNER, J. SEEB, AND L. WISHARD. 1980. Population structure of indigenous salmonid species of the Pacific Northwest, p. 285–304. *In* W. McNeil and D. Himsworth [ed.] Salmonid ecosystems of the North Pacific. University of Oregon Press, Corvallis, OR.

VANSTONE, W. E., AND J. R. MARKERT. 1968. Some morphological and biochemical changes in coho salmon (*Oncorhynchus kisutch*) during parr–smolt transformation. J. Fish. Res. Board Can. 25: 2403–2418.

WEBB, P. W. 1977. Effects of median fin amputation on fast-start performance of rainbow trout. J. Exp. Biol. 68: 123–135.

1978. Fast-start performance and body form in seven species of teleost fish. J. Exp. Biol. 74: 211–226.

WILCOCK, J. A., AND D. N. MCBRIDE. 1983. Origins of chinook salmon (*Oncorhynchus tshawytscha*) in the Yukon River fisheries, 1982. Alaska Dep. Fish Game Inf. Leafl. No. 226: 36 p.

YEVSIN, Y. N. 1976. Morphological characteristics and variability of local stocks of the autumn sea trout (*Salmo trutta*) of the rivers of the White Sea Basin. J. Ichthyol. 16: 911–921.

1977. Morphological characteristics and variability of the summer sea trout (*Salmo trutta*) from the Pulmg'a and Malaya Kumzhevaya rivers. J. Ichthyol. 17: 350–356.

Variation in Burst and Prolonged Swimming Performance Among British Columbia Populations of Coho Salmon, *Oncorhynchus kisutch*

Eric B. Taylor and J. D. McPhail

Institute of Animal Resource Ecology and Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 1W5

Taylor, E. B., and J. D. McPhail. 1985. Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42: 2029–2033.

Burst swimming tests, using both wild and laboratory-reared juvenile coho salmon, *Oncorhynchus kisutch*, from three streams (two south coastal British Columbia and one interior), indicated that in the first few hundredths of a second of a "fast-start" the coastal juveniles attain greater burst velocities than similar sized interior juveniles. Interior (Coldwater River) juveniles had greater swimming stamina than individuals from the two coastal streams: Wade and Morrison creeks. The differences in stamina performance were demonstrated to be inherited. The differences in swimming performance between coastal and interior are related to differences in body form; coastal coho have deep, robust bodies, while interior juveniles are more fusiform. The differences in swimming performance between coastal and interior coho salmon are probably adaptive and related to differences in the energetic demands of their freshwater migrations and perhaps to different levels of predation in coastal and interior streams.

Des essais de départ rapide portant sur des saumons cohos (*Oncorhynchus kisutch*) juvéniles sauvages ou élevés en laboratoire, originaires de trois cours d'eau (deux cours d'eau de la côte sud et un de l'intérieur de la Colombie-Britannique), ont montré que, au cours des premiers centièmes de seconde d'un départ rapide, les poissons juvéniles côtiers atteignaient une plus grande vitesse que ceux, de taille similaire, originaires des eaux intérieures. Ces derniers (rivière Coldwater) avaient plus d'endurance que les poissons des deux cours d'eau côtiers (ruisseaux Wade et Morrison). Il a été démontré que cet écart d'endurance était héréditaire. Les différences de rendement de nage entre les deux types de poisson étaient fonction de la configuration corporelle : les cohos côtiers ont un corps épais et massif tandis que celui des poissons de l'intérieur est plus fusiforme. Les écarts de rendement de nage des deux types de poisson sont probablement d'origine adaptative et fonction de la demande énergétique des migrations en eau douce; ils découlent peut-être aussi de niveaux de prédation différents entre les eaux côtières et intérieures.

Received April 29, 1985
Accepted August 12, 1985
(J8221)

Reçu le 29 avril 1985
Accepté le 12 août 1985

Despite the long history of morphological "stock identification" studies in various fish species (Hill 1959; Wilk et al. 1980; Ihssen et al. 1981b; Meng and Stockner 1984), little is known of the functional significance of interpopulation differences in morphology. Such information, however, is important when evaluating the role of selection in defining local populations and represents a major void in existing fisheries knowledge (Ihssen et al. 1981a). We have explored (Taylor and McPhail 1985a) the body morphology of south coast – Fraser River coho salmon, *Oncorhynchus kisutch*, and documented inherited differences in body form between coastal and interior coho. Both juvenile and adult coastal coho (from streams tributary to the Strait of Georgia and lower Fraser River) are more robust bodied (deeper, wider bodies, greater median fin areas) than interior coho (upstream of Hell's Gate in the Fraser River system). Since it has often been suggested that body form may be significant in swimming mode and performance (Breder 1926; Kanep 1971; Weihs 1973; Webb 1977, 1978a; Riddell and Leggett 1981), we wondered if body form differences between coastal and interior coho salmon were linked to differences in swimming performance. Further, the native distribution of coho in the northeastern Pacific includes both coastal (west of the Coast Range) and interior (east of the Coast Range) anadromous populations (Lee et al. 1980; Fraser et al. 1982). Thus, spawning and smolt migrations may extend from less than 1 km in small coastal streams to over 1000 km in the upper Fraser and Columbia rivers. These differences in distance, and perhaps difficulty, of migration suggest that interior and coastal coho populations may be under different selective regimes relative to prolonged swimming performance.

Our objectives in this study were to (1) assess burst and prolonged swimming performance in juvenile coho salmon and (2) determine if any differences in swimming performance were in a direction consistent with the differences in body form between coastal and interior coho, as suggested by hydromechanical theory. Specifically, deep, robust bodies are considered to yield high burst swimming performance (Weihs 1973; Webb 1977, 1978a) and thus we predicted that coastal coho

would be better at burst swimming. Fusiform bodies may reduce swimming drag (Webb 1975) and we predicted that interior coho would be better at prolonged swimming performance.

Materials and Methods

Our experiments involved wild and laboratory-raised juveniles from the Coldwater River (an interior river located in the Thompson River drainage), Morrison Creek (east coast of Vancouver Island), and Wade Creek (lower Fraser River). The wild fish were collected using minnow traps and seines. Eggs and milt were collected from spawning adults from the three populations and reared in the laboratory, as outlined by Taylor and McPhail (1985a). Fish were deprived of food for 24 h before all swimming tests.

Burst Swimming Tests

Test fish ranged from 4.0 to 7.0 cm standard length, with approximately equal numbers of fish in the three length categories: 4.0–5.0, 5.1–6.0, and 6.1–7.0 cm. There were no differences in mean standard length among the test fish from the different populations (analysis of variance, minimum $p > 0.05$). Burst swimming tests on fry were conducted in a test tank and swimming sequences were recorded with a high-speed movie camera, as described by Taylor and McPhail (1985b). To initiate burst swimming in the test fish an electrical stimulus ($1.5\ V \cdot cm^{-1}$) was delivered through electrodes placed in the test tank. Fish were tested only once. Water temperature was maintained at 13.5 ± 1.0°C and the water level at 8.0 cm. The film was exposed at 100 frames$\cdot s^{-1}$, f2.8, and the processed film was analysed frame-by-frame on a motion analyzer (Photographic Analysis Ltd.).

Spatial displacement over time was taken directly from the filmed sequences. By employing the framing rate as the time variable, velocity calculations were then made from these data. Velocities for each fish were calculated at 0.01-s time intervals. We used mean and maximum velocities attained per burst as criteria to assess burst swimming performance, since displacement over time is the best overall measure of performance (Webb 1978b). Mean velocity per burst was calculated for each fish from the velocities at individual 0.01-s time intervals and then pooled by population. Maximum velocities attained per burst were calculated for all fish and then pooled by population. These data formed the basis for mean and maximum velocity versus standard length regressions (Webb and Corolla 1981). Repeated analysis of fish from the film data indicated maximum error rates of about 10% for maximum velocity per burst, and less than 5% for mean velocity.

Stamina Swimming Tests

Stamina tests were conducted in an apparatus described previously by Tsuyuki and Williscroft (1977). The system was modified slightly by placing two No. 20 mesh (0.9 × 0.9 mm) stainless steel turbulence screens, separated by 4.0 cm, at the upstream end of the tunnels. This modification was necessary to prevent strong rotational water flow at the head of each tunnel.

When the coho salmon fry reached a standard length of about 5.0 cm they were taken for training and testing. At this point, 30 individuals from a population were placed in 210-L oval training tanks. To bring all test fish up to some minimum level of swimming performance, fish were trained to swim at 2.5 bodylengths$\cdot s^{-1}$ for a period of 2 wk. During training, fish were fed three times per day. Stamina swimming performance was measured using the "fixed-velocity" method (Brett 1967; Beamish 1978). This method measures the time required for fish to fatigue when swimming at a constant water velocity. After the 2-wk training period, 12 fish from each population were placed in the stamina tubes and allowed to acclimate to the tunnels for 2 h. At this time the water velocity was maintained at the "training velocity" (2.5 bodylengths$\cdot s^{-1}$). To encourage swimming in the test fish, a downstream electric field (5.0 V, AC) was activated. After 2 h, the water velocity in the tunnels was increased, over 1 min, to one half the test velocity and then left for 30 min. Finally, the water velocity was increased to the test velocity (hereafter referred to as V_t) and the time was recorded.

Pilot experiments indicated that a V_t of 7.0 bodylengths$\cdot s^{-1}$ was needed to induce fatigue in all populations. Fatigue was defined as the point when a fish was impinged against wire screening at the rear of the stamina tubes and could not be induced to continue swimming with gentle proding. As each fish fatigued over the course of a test, the time to fatigue was noted. The fish was then removed from the tunnel and its standard length recorded. To reduce the intrapopulation variability in swimming performance and to test the "best" individuals of each sample, only those individuals that could maintain position for at least 1 min at V_t were included in our analysis. Two populations were tested at a time, each population in a separate tunnel, and each population was tested three times. To eliminate possible tunnel bias each population was tested in all tunnels over the course of the tests. The tunnels were covered with black polyethylene during tests. Swimming fish were periodically checked in the tunnels by looking into a mirror positioned at 45° along the side of each tunnel. The water temperature in the tunnels was maintained at 11.5 ± 1.0°C. Water velocities were measured with an Ott current meter. Oxygen levels were monitored periodically and in all cases remained above 95% saturation throughout the tests. Each test lasted a minimum of 360 min (see Brett 1983) or until at least 50% of the individuals fatigued. All tests were conducted in June, July, and August of 1983.

Statistical Methods

In the population burst tests, regressions were calculated between both maximum and mean velocity per burst and standard length. Differences in slopes between groups were tested using covariance analysis (ANCOVA), and in only one comparison of two slopes was there a significant difference ($p < 0.05$). Thus, both mean and maximum velocities attained by individuals in each population were adjusted to a common standard length and were compared by ANCOVA.

In the stamina experiments, regressions were calculated between probits of cumulative percent fatigued and the logarithms (base 10) of individual fatigue times (Finney 1971; Tsuyuki and Williscroft 1977). Preliminary estimates of population median fatigue times and the distributional standard deviations were derived from this analysis (Bliss 1937). In many tests, particularly those involving Coldwater River coho, only a proportion of the fish fatigued during a test. In such cases the distribution of fatigue times was truncated. Thus, the preliminary estimates of the response statistics were used to complete final solutions for such truncated distributions using the methods outlined in Johnson and Kotz (1970). The antilog of the time at which 50% of test fish from a single population

TABLE 1. Adjusted (to 5.5 cm standard length) mean and maximum velocities ($cm \cdot s^{-1}$) attained per burst from ANCOVA analysis, with standard length as the covariate, for wild and laboratory-reared juvenile coho salmon from the three study populations. Underlined mean values differ significantly from each other (ANCOVA, $p < 0.05$).

	Adjusted V_{mean}			Adjusted V_{max}	
Group	Mean	SD	N	Mean	SD
Wild					
Coldwater River	62.9[a]	6.07	30	102.3	13.87
Morrison Creek	71.8[a]	11.96	28	104.7	12.31
Wade Creek	74.1	7.28	30	104.7	11.18
Laboratory-reared					
Coldwater River	58.8	7.77	29	90.7	10.51
Morrison Creek	66.1	9.26	30	97.9	13.45
Wade Creek	66.1	5.03	30	102.1	16.15

[a]Slopes of standard length – velocity not equal between these groups ($p < 0.05$).

fatigued (i.e. the FT_{50}) was used as an index of swimming stamina (Brett 1967). We used "approximate" one-way analysis of variance (Sokal and Rohlf 1969) to test for differences among sample FT_{50}'s. The calculation of the approximate F statistic (F') does not assume homogeneity of sample variances and the means are weighted based on the reciprocal of the variance of the sample from which each mean was derived.

Results

Burst Swimming Tests

The coastal juvenile coho consistently attained greater mean and maximum velocity during burst swimming, although in only one comparison was the difference significant (V_{max}, WC lab > CW lab, $p < 0.025$, Table 1). In velocities attained during burst swimming the populations consistently ranked Wade > Morrison > Coldwater in both wild and laboratory-reared juveniles (Table 1). This ranking of burst swimming performance is in the same order as the ranking of body robustness of juveniles from these populations (Taylor and McPhail 1985a). Thus, the most robust-bodied coho consistently attained the highest burst velocities (Table 1).

Maximum velocity – standard length data were pooled into "coastal" and "interior" groups and ANCOVA performed on the pooled data. The slopes were equal ($p > 0.05$), and the adjusted V_{max} (to 5.4 cm) was higher in the coastal sample (102.3 vs. 95.5 $cm \cdot s^{-1}$). This difference, however, was not significant ($p > 0.05$).

Stamina Swimming Tests

In both wild and laboratory-reared juveniles, Coldwater River (interior) fish had greater swimming stamina (FT_{50}) than either of the two coastal populations (Tables 2 and 3). For the Coldwater fish the FT_{50} values were about 3.5 times those for Wade Creek fish and over 7 times those of Morrison Creek wild fish (maximum $p < 0.001$). In only two cases (Morrison Creek, $0.001 < p < 0.01$) were there significant differences in FT_{50} values within a population. In contrast, all Coldwater River FT_{50} values were higher (maximum $p < 0.01$) than those of Morrison and Wade Creek wild samples, and all Wade Creek sample FT_{50}'s were greater than those for Morrison Creek

TABLE 2. Prolonged swimming performance of wild-reared juvenile coho salmon from the three study populations.

	Standard length (cm)			Test velocity,	FT_{50}	
Population	Mean	SD	N	($cm \cdot s^{-1}$)	(min)	SD
Coldwater River	4.92	0.134	12		63.5	14.6
Wade Creek	5.07	0.122	11	34.3	28.3	9.1
Coldwater River	4.96	0.162	10		79.9	36.03
Morrison Creek	5.03	0.166	12	34.7	9.2	3.58
Wade Creek	5.13	0.143	12		29.4	3.82
Morrison Creek	5.06	0.198	12	35.4	9.9	4.73
Coldwater River	5.05	0.161	11		79.9	11.46
Morrison Creek	5.22	0.132	11	35.7	16.7	5.16
Wade Creek	5.08	0.218	11	35.5	33.4	7.36

TABLE 3. Prolonged swimming performance of laboratory-reared juvenile coho salmon from the three study populations.

	Standard length (cm)			Test velocity,	FT_{50}	
Population	Mean	SD	N	($cm \cdot s^{-1}$)	(min)	SD
Coldwater River	5.17	0.202	10		105.9	10.4
Morrison Creek	5.20	0.170	11	36.1	9.1	4.6
Coldwater River	5.38	0.208	10		80.5	10.3
Wade Creek	5.33	0.136	10	37.1	28.5	6.5
Wade Creek	5.18	0.114	10		28.8	10.1
Morrison Creek	5.11	0.127	11	36.1	8.9	5.1
Coldwater River	5.22	0.211	11		104.2	40.8
Wade Creek	5.37	0.221	9	36.7	9.2	3.8
Morrison Creek	5.38	0.208	11	37.5	16.6	5.2

(p range 0.01–0.001). In laboratory-reared juveniles (Tabie 3), Coldwater River coho FT_{50}'s averaged 4.3 times those of Wade Creek fish and over 8 times those of Morrison Creek samples ($p < 0.001$). Among the laboratory tests there was at least one significant difference for within-population comparisons for all three populations (maximum $p < 0.01$). Coldwater River juvenile coho, however, again had greater FT_{50}'s than Wade and Morrison Creek juveniles (maximum $p < 0.001$). Wade Creek FT_{50}'s were greater than Morrison Creek FT_{50}'s in six of nine tests (maximum $p < 0.001$), while their FT_{50}'s for two tests were no different and in one case the Morrison Creek FT_{50} was greater than that for Wade Creek (16.6 vs. 9.2 min, $p < 0.01$). In summary, for both wild and laboratory-reared juveniles, the populations consistently ranked Coldwater River > Wade Creek > Morrison Creek in prolonged swimming performance.

Discussion

Coho salmon juveniles from the interior are apparently well adapted for prolonged swimming. Coldwater River (interior)

coho juveniles had, on average, four to five times the prolonged swimming performance of coastal juveniles. Since these differences were maintained in juveniles reared from eggs in the laboratory under identical conditions, they are probably largely inherited. Wild-reared juvenile coho from Louis Creek, a tributary to the North Thompson River near Heffley Creek, British Columbia, also have greater prolonged swimming performance than coho from coastal creeks (E. B. Taylor, unpubl. data). Interpopulation differences in stamina swimming performance in coho yearlings were demonstrated by Besner and Smith (1983) and they suggested that the differences were genetic in origin. Tsuyuki and Williscroft (1977) demonstrated that laboratory-reared steelhead trout, *Salmo gairdneri*, juveniles from the Thompson River (upper Fraser River) had greater swimming stamina than juveniles from the Vedder River (lower Fraser River). The greater prolonged swimming performance of the Thompson River steelhead was correlated with higher levels of the LDH-A allele. This allele has been functionally associated, through elevation of muscular fatigue thresholds, with increased prolonged swimming ability (Tsuyuki and Williscroft 1977). Thus, the increased prolonged swimming performance of both upper Fraser steelhead and coho (this study) appears to be an adaptation to the long and energetically more costly (Idler and Clemens 1959; Gilhousen 1980) migration routes of these populations.

Distance and/or difficulty of freshwater migrations have been considered by many as powerful selective factors acting on anadromous fishes, especially salmonids (Scott 1962; Banks 1969; Ricker 1972; Schaffer and Elson 1975; Glebe and Leggett 1976; Leggett and Carscadden 1978; Power 1981; Thorpe and Mitchell 1981; Healey 1983). This is certainly true of the Fraser River system where specific velocity barriers encountered in migration (e.g. Hell's Gate in the Fraser Canyon, rapid sections of Thompson River) in addition to the length of migration have historically represented significant hydrodynamic barriers to fish passage (Ricker 1950, 1972). The selective nature of (1) distance and (2) rapid water may be intensified by the fact that the upstream migration is a spawning migration. Certainly, delays in migration or situations that increase energy expenditure on route are known to reduce spawning success (Ricker 1950; Idler and Clemens 1959; Geen 1975).

Several factors may contribute to the superior prolonged swimming ability of interior coho, including body morphology. Interior coho salmon have a more fusiform body shape than coastal coho, a difference that is also inherited (Taylor and McPhail 1985a). The fusiform body shape of interior coho may increase migration success by reducing swimming drag. A few other studies involving salmonids have noted that among life history stages or populations within a species, those groups under what are interpreted as greater hydrodynamic constraints (long migrations, high water velocity) are characterized by body forms proposed to impart some hydrodynamic advantage (fusiform bodies, longer paired fins (Evropeitseva 1962; Vanstone and Markert 1968; Fessler and Wagner 1969; Yevsin 1976, 1977; Riddell and Leggett 1981)).

Our burst swimming tests also suggest that body form is related to swimming performance; the robust-bodied coastal juvenile coho were better at burst swimming than interior juveniles (Table 1). However, causal relationships between body form and swimming performance cannot be established from our data. Physiological and enzymatic differences are but two other factors that are known to be related to swimming performance (Tsuyuki and Williscroft 1977; Beamish 1978). Only when swimming performance, body form, and physiological and enzymatic parameters are measured concurrently in a single study can the nature of the relationships between body form and swimming performance be properly assessed.

High burst swimming performance and robust bodies may represent alternative adaptations in coastal juvenile coho. In fishes, morphological responses to differential predation pressure have been suggested by various workers (Larson 1976; Moodie and Reimchen 1976; McPhail 1977; Reimchen 1980; Reist 1980a, 1980b). Further, burst swimming performance appears to be directly related to the ability to evade predators (Webb 1981; Webb and Corolla 1981; Taylor and McPhail 1985b), and body shape is an important variable in defining levels of evasion performance (Weihs 1973; Webb 1977, 1978a). Since coastal streams are close to estuaries, they may have a greater diversity of predators than interior streams, primarily through the inclusion of certain euryhaline fish species and because these streams are near coastal migration routes of many piscivorous birds (Munro and Cowan 1947; Mace 1983). Further, coastal streams may be subject to large inputs of predatory smolt and adult salmonids (Lagler and Wright 1962; Meacham and Clark 1979). The results of experiments involving newly emerged coho salmon fry (Taylor and McPhail 1985b) and results documented by Patten (1975, 1977) demonstrate that larger, more robust-bodied juvenile salmonids have reduced susceptibility to predation. Consequently, the greater body depth and width of coastal coho may reduce their susceptibility to predators, either through increased burst swimming performance or increased difficulty in manipulation and swallowing (Mace 1983).

In summary, we have found that coastal and interior coho are very different with respect to aspects of swimming performance, differences with a genetic component and which we suggest are adaptive. Differences in body form between coastal and interior coho salmon are also inherited and may contribute to the observed differences in swimming performance.

Acknowledgments

We appreciate the field assistance provided by G. Birch, S. Cox-Rogers, and P. Lavin. P. A. Larkin, R. W. Blake, and P. Lavin made helpful critisicms during the course of this research. We thank T. Howard of the British Columbia Research Council and R. W. Blake for the loan of the stamina and camera equipment, respectively. Funding was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) strategic grant (67-0976).

References

BANKS, J. W. 1969. A review of the literature on the upstream migration of adult salmonids. J. Fish Biol. 1: 85–136.

BEAMISH, F. W. 1978. Swimming capacity, p. 101–187. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Volume VII. Academic Press Ltd, New York, NY.

BESNER, M., AND L. S. SMITH. 1983. Modification of swimming mode and stamina in two stocks of coho salmon (*Oncorhynchus kisutch*) by differing levels of long-term continuous exercise. Can. J. Fish. Aquat. Sci. 40: 733–739.

BLISS, C. I. 1937. The calculation of the time–mortality curve. Ann. Appl. Biol. 24: 815–852.

BREDER, C. M. 1926. The locomotion of fishes. Zoologica (N.Y.) 4: 159–297.

BRETT, J. R. 1967. Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. J. Fish. Res. Board Can. 24: 1731–1741.

1983. The swimming speed of adult pink salmon, *Oncorhynchus gorbuscha*, at 20°C and a comparison with sockeye salmon, *O. nerka*. Can. Tech. Rep. Fish. Aquat. Sci. 1143: 37 p.

EVROPEITSEVA, N. V. 1962. Comparative analysis of the desmoltification process among the young of different ecological forms of Atlantic salmon. Uch. Zap. Leningr. Gos. Univ. 311: 46–73. (Transl. from Russian by Fish. Res. Board Transl. Ser. 431)

FESSLER, J. L., AND H. F. WAGNER. 1969. Some morphological and biochemical changes in steelhead trout during the parr–smolt transformation. J. Fish. Res. Board Can. 26: 2823–2841.

FINNEY, D. J. 1971. Probit analysis. 3rd ed. Cambridge University Press, Cambridge, England. 318 p.

FRASER, F. J., A. Y. FEDORENKO, AND P. J. STARR. 1982. Review of the chinook and coho of the Fraser River. Can. Tech. Rep. Fish. Aquat. Sci. 1126: 130 p.

GEEN, G. H. 1975. Ecological consequences of the proposed Moran Dam on the Fraser River. J. Fish. Res. Board Can. 32: 126–135.

GILHOUSEN, P. 1980. Energy sources and expenditures in Fraser River sockeye salmon during their spawning migration. Int. Pac. Salmon Fish Comm. Bull. 22: 51 p.

GLEBE, B. D., AND W. C. LEGGETT. 1976. Weight loss and energy expenditure of American shad during the freshwater migration. Final Rep. Prog. AFC-8, U.S. Natl. Mar. Fish. Serv.

HEALEY, M. C. 1983. Coastwide distribution and ocean migration patterns of stream- and ocean-type chinook salmon, *Oncorhynchus tshawytscha*. Can. Field-Nat. 97: 427–433.

HILL, D. R. 1959. Some uses of statistical analysis in classifying races of American shad (*Alosa sapidissima*). Fish. Bull. 59: 269–286.

IDLER, D. R., AND W. A. CLEMENS. 1959. The energy expenditure of Fraser River sockeye salmon during migration, spawning and death. Int. Pac. Salmon Fish. Comm. Prog. Rep. 6: 80 p.

IHSSEN, P. E., H. E. BOOKE, J. M. CASSELMAN, J. M. MCGLADE, N. R. PAYNE, AND F. M. UTTER. 1981a. Stock identification: materials and methods. Can. J. Fish. Aquat. Sci. 38: 1838–1855.

IHSSEN, P. E., D. O. EVANS, W. J. CHRISTIE, J. A. RECKAHN, AND R. L. DESJARDINE. 1981b. Life history, morphology, and electrophoretic characteristics of five allopatric stocks of lake whitefish (*Coregonus clupeaformis*) in the Great Lakes region. Can. J. Fish. Aquat. Sci. 38: 1790–1807.

JOHNSON, N. L., AND S. KOTZ. 1970. Distributions in statistics. 1. Continuous univariate distributions. Houghton Mifflin, New York, NY. 338 p.

KANEP, S. V. 1971. Some aspects of the functional morphology of the lake pelyad (*Coregonus peled* [Gmelin]). Vopr. Ikhtiol. 11: 855–866.

LAGLER, K., AND A. T. WRIGHT. 1962. Predation by Dolly Varden, *Salvelinus malma*, on young salmon, *Oncorhynchus* spp., in an estuary in southeast Alaska. Trans. Am. Fish. Soc. 91: 90–93.

LARSON, G. E. 1976. Social behaviour and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake. Can. J. Zool. 54: 107–121.

LEE, D. S., C. R. GILBERT, C. H. HOCUTT, R. E. JENKINS, D. E. MCALLISTER, AND J. R. STAUFFER JR. 1980. Atlas of North America freshwater fishes. N. C. Biol. Surv. Publ. No. 1980-12: 854 p.

LEGGETT, W. C., AND J. E. CARSCADDEN. 1978. Latitudinal variation in reproductive characteristics of American shad (*Alosa sapidissima*): evidence for population specific life history strategies in fish. J. Fish. Res. Board Can. 35: 1469–1478.

MACE, P. M. 1983. Bird predation on juvenile chum salmon in the Big Qualicum River estuary. Appendix 1. Doctoral dissertation, University of British Columbia, Vancouver, B.C. 644 p.

MCPHAIL, J. D. 1977. Inherited differences in the size at first spawning among sticklebacks. Heredity 38: 53–60.

MEACHAM, C. P., AND J. H. CLARK. 1979. Management to increase anadromous salmon production, p. 377–385. *In* H. Clepper and R. Stroud [ed.] Predator–prey systems in fisheries management. Sport Fishing Institute Spec. Publ., Washington, DC. 504 p.

MENG, H. J., AND M. STOCKNER. 1984. An evaluation of morphometrics and meristics for stock separation of Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 41: 414–422.

MOODIE, G. E. E., AND T. E. REIMCHEN. 1976. Phenetic variation and habitat differences in (*Gasterosteus*) populations of the Queen Charlotte Islands. Syst. Zool. 25: 49–61.

MUNRO, J. A., AND I. MCT. COWAN. 1947. A review of the bird fauna of British Columbia. British Columbia Prov. Mus. Spec. Publ. No. 2. Victoria, B.C. 285 p.

PATTEN, B. G. 1975. Comparative vunerability of Pacific salmon and steelhead trout to predation by torrent sculpin in stream aquaria. Fish. Bull. 73: 931–934.

1977. Body size and learned avoidence as factors affecting predation on coho salmon, *Oncorhynchus kisutch*, fry by torrent sculpin, *Cottus rotheus*. Fish. Bull. 75: 457–459.

POWER, G. 1981. Stock characteristics and catches of Atlantic salmon (*Salmo salar*) in Quebec, and Newfoundland and Labrador in relation to environmental variables. Can. J. Fish. Aquat. Sci. 38: 1601–1611.

REIMCHEN, T. E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: and adaptation to predators? Can. J. Zool. 58: 1232–1244.

REIST, J. D. 1980a. Selective predation upon phenotypes of brook stickleback, *Culaea inconstans*, by northern pike, *Esox lucius*. Can. J. Zool. 58: 1245–1258.

1980b. Predation upon pelvic phenotypes of brook stickleback, *Culaea inconstans*, by selected invertebrates. Can. J. Zool. 58: 1253–1258.

RICKER, W. E. 1950. Cyclic dominance among Fraser River sockeye. Ecology 31: 6–26.

1972. Hereditary and environmental factors affecting certain salmonid populations, p. 19–160. *In* R. C. Simon and P. A. Larkin [ed.] The stock concept in Pacific salmon. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C. 231 p.

RIDDELL, B. E., AND W. C. LEGGETT. 1981. Evidence for an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*) Can. J. Fish. Aquat. Sci. 38: 308–320.

SCHAFFER, W. M., AND P. F. ELSON. 1975. The adaptive significance of variations in life history among local populations of Atlantic salmon in North America. Ecology 56: 577–590.

SCOTT, D. P. 1962. Effect of food quantity on fecundity of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 19: 715–731.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freeman and Co., San Francisco, CA. 776 p.

TAYLOR, E. B., AND J. D. MCPHAIL. 1985a. Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42: 2020–2028.

1985b. Burst swimming and size-related predation in newly emerged coho salmon, *Oncorhynchus kisutch*. Trans. Am. Fish. Soc. 114: 546–551.

THORPE, J. E., AND K. A. MITCHELL. 1981. Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness and current management. Can. J. Fish. Aquat. Sci. 38: 1576–1590.

TSUYUKI, H., AND S. N. WILLISCROFT. 1977. Swimming stamina differences between genotypically distinct forms of rainbow trout (*Salmo gairdneri*) and steelhead trout. J. Fish. Res. Board Can. 34: 996–1003.

VANSTONE, W. E., AND J. R. MARKERT. 1968. Some morphological and biochemical changes in coho salmon (*Oncorhynchus kisutch*) during parr–smolt transformation. J. Fish. Res. Board Can. 25: 2403–2418.

WEBB, P. W. 1975. Hydromechanics and energetics of fish propulsion. Bull. Fish. Res. Board Can. 190: 158 p.

1977. Effects of median fin amputation on fast-start performance of rainbow trout. J. Exp. Biol. 68: 123–135.

1978a. Fast-start performance and body form in seven species of teleost fish. J. Exp. Biol. 74: 211–266.

1978b. Temperature effects on acceleration performance of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 35: 1417–1422.

1981. Responses of northern anchovy, *Engraulis mordax*, larvae to predation by a biting planktivore, *Amphiprion percula*. Fish. Bull. 79: 727–735.

WEBB, P. W., AND T. R. COROLLA. 1981. Burst swimming performance of northern anchovy, *Engraulis mordax* larvae. Fish. Bull. 79: 143–150.

WEIHS, D. 1973. The mechanism of rapid starting of a slender fish. Biorheology 10: 343–350.

WILK, S. J., W. G. SMITH, D. E. RAIPH, AND J. SIBUNKA. 1980. Population structure of summer flounder between New York and Florida based on linear discriminant analysis. Trans. Am. Fish. Soc. 109: 265–271.

YEVSIN, Y. N. 1976. Morphological characteristics and variability of local stocks of the autumn sea trout (*Salmo trutta*) of the rivers of the White Sea Basin. J. Ichthyol. 16: 911–921.

1977. Morphological characteristics and variability of the summer sea trout (*Salmo trutta*) from the Pulmg'a and Malaya Kumzhevaya rivers. J. Ichthyol. 17: 350–356.

Acidification and Toxicity of Metals to Aquatic Biota

P. G. C. Campbell

Université du Québec, INRS-Eau, C.P. 7500, Sainte-Foy, Qué. G1V 4C7

and P. M. Stokes

University of Toronto, Institute for Environmental Sciences, Haultain Building, Toronto, Ont. M5S 1A4

Campbell, P. G. C., and P. M. Stokes. 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. 42: 2034–2049.

Acidification of freshwaters can in principle influence metal–organism interactions in at least two ways: the decrease in pH may affect metal speciation in solution, or it may affect biological sensitivity at the level of the cell surface. We considered the possible effects of environmental acidification (pH 7–4) on (1) metal speciation in solution, (2) metal adsorption at biological surfaces, and (3) metal uptake by and toxicity to aquatic biota. Attention was focused on some 10 metals of potential concern in the context of freshwater acidification (Ag, Al, Cd, Co, Cu, Hg, Mn, Ni, Pb, Zn). For the four metals (Al, Cu, Hg, Pb) predicted to manifest speciation changes in the range pH 7–4, confirmatory experimental data are available for two (Cu, Pb). In the six remaining cases predicted to show little sensitivity to pH changes in this range, supporting experimental evidence exists for four metals (Ag, Cd, Mn, Zn). A pH-dependent biological response is documented over a realistic range of H^+ and metal concentrations for 6 of the 10 metals considered (Al, Cd, Cu, Zn, and to a lesser extent, Hg and Pb). These six metals fall into two groups: those for which a decrease in pH results in a decreased biological response (type I behavior: Cd, Cu, Zn) and those for which the dominant effect of acidification is to increase metal availability (type II behavior: Pb). Data for the remaining two metals (Al, Hg) clearly reveal pH effects, but the results are too few and too inconsistent to allow generalizations.

L'acidification des eaux douces peut, en principe, influer sur les interactions entre les organismes et les métaux d'au moins deux façons : la diminution du pH peut modifier la forme sous laquelle se présentent les métaux en solution ou la réponse biologique au niveau de la surface de la cellule. Nous avons examiné les effets possibles de l'acidification de l'environnement (variation du pH de 7 à 4) sur : (1) la forme que présentent les métaux en solution, (2) l'absorption des métaux au niveau des surfaces biologiques, et (3) l'absorption de métaux par les organismes aquatiques et la toxicité que cela représente pour ces derniers. L'accent a été mis sur quelques dix métaux pouvant susciter des inquiétudes dans le cadre de l'acidification des eaux douces (Ag, Al, Cd, Co, Cu, Hg, Mn, Ni, Pb, Zn). En ce qui concerne les quatre métaux (Al, Cu, Hg, Pb) censés montrer des changements de forme dans la gamme de pH allant de 7 à 4, on possède des données expérimentales qui confirment ce fait pour deux d'entre eux, soit le Cu et le Pb. Dans les six autres cas où l'on prévoyait peu de réaction aux changements de pH dans cette échelle de valeurs, il existe des preuves expérimentales à l'appui pour quatre métaux (Ag, Cd, Mn, Zn). Une réponse biologique en fonction du pH est établie, couvrant une gamme réaliste de concentrations de métaux et de H^+ pour 6 des 10 métaux envisagés (Al, Cd, Cu, Zn et, à un moindre degré, Hg et Pb). Ces six métaux tombent dans deux catégories : ceux pour lesquels une diminution de pH se traduit par une reponse biologique réduite (comportement de type I : Cd, Cu, Zn) et ceux pour lesquels l'effet principal de l'acidification est d'accroître la disponibilité du métal (comportement de type II : Pb). Les données concernant les deux autres métaux (Al, Hg) montrent clairement les effets du pH, mais les résultats sont trop peu nombreux et pas assez cohérents pour permettre des généralisations.

Received April 15, 1985
Accepted July 30, 1985
(J8186)

Reçu le 15 avril 1985
Accepté le 30 juillet 1985

With the progress that has been made in aquatic toxicology over the past 10–15 yr, there is wide recognition that a particular metal in water can exist in more than one form, and that different forms have different availabilities to living organisms. It is now axiomatic that a value for total metal in a given water sample is rarely adequate to predict even in general terms the potential toxicity of the water. In fact, there is a steadily increasing body of evidence, derived from laboratory experiments with a variety of aquatic organisms (e.g. algae, bacteria, microcrustacea, fish), demonstrating that the biological response elicited by a dissolved metal is usually a function of the free metal ion concentration, $M^{z+}(H_2O)_n$, which in turn is determined not

only by the total dissolved metal concentration but also by the concentration and nature of the ligands present (Borgmann 1983).

Metal complexes with organic and inorganic ligands tend to dissociate as pH decreases, resulting in increased free ion concentrations. This trend has led to the general acceptance of the idea that metals are more likely to be toxic to biota in acidic than in neutral waters. This is indirectly supported by the generally low species richness, low productivity, and small standing crop of biota in acidic waters. So compelling was the case made by the chemical speciation approach that acceptance of the idea that "more acid" means "more metal toxicity" seems not to have been questioned, let alone challenged, and certainly not tested, until quite recently.

Here we review anticipated and observed variations in metal bioavailability and toxicity over the pH range 7–4. Our emphasis is accorded not to changes in *total* dissolved metal concentration (i.e. geochemical mobilization), but rather to possible changes in metal speciation occasioned by the decrease in pH and to concomitant changes in biological susceptibility over this pH range.

Theoretical Considerations

Influence of pH on Metal Speciation in Solution

Even at constant dissolved metal concentration, changes in metal speciation can be anticipated as a result of a decrease in pH, e.g.:

a shift in the hydrolysis equilibrium to the left, favoring the aquo ion:

$$(1)\quad M^{z+}(H_2O)_n \overset{K_1}{\rightleftarrows} M(H_2O)_{n-1}(OH)^{(z-1)+} + H^+$$

a shift in complexation equilibria (competition between M^{z+} and H^+ for the same ligands):

$$(2)\quad M^{z+} + nL \overset{K_2}{\rightleftarrows} ML_n^{z+}$$

$$(3)\quad H^+ + L \overset{K_3}{\rightleftarrows} HL^+$$

where M^{z+} = generalized metal cation of charge $+z$ (shown without its hydration sphere for simplicity) and L = ligand (shown uncharged for simplicity). The degree to which the speciation of a particular metal is affected by a decrease in pH will depend on the acid–base properties of L and on the stability of the various ML species.

Influence of pH on Metal–Surface Interactions at the Cell–Medium Interface

To elicit a biological response from a target organism and/or to accumulate within this organism, a given metal must obviously interact with/traverse a cell membrane. This interaction of the metal with the cell surface, normally involving either M^{z+} or ML_n^{z+} as the reactive species, can be represented in terms of the formation of M–X–cell surface complexes (Nelson et al. 1981; Petersen 1982; Morel and Morel-Laurens 1983; Sunda and Huntsman 1983), where X–cell = a cellular ligand and *the biological response is proportional to* $[M^{z+}–X–cell]$

$$(4)\quad M^{z+} + X\text{–cell} \overset{K_4}{\rightleftarrows} M^{z+}\text{–X–cell}$$

$$(4')\quad [M^{z+}\text{–X–cell}] = K_4[X\text{–cell}][M^{z+}]$$

$$(5)\quad ML_n^{z+} + X\text{–cell} \overset{K_5}{\rightleftarrows} M^{z+}\text{–X–cell} + nL.$$

In the latter case (equation (5)), if equilibrium conditions are assumed, the concentration of cell-bound metal can be expressed as

$$(6)\quad [M^{z+}\text{–X–cell}] = K_5 \frac{[X\text{–cell}][ML_n^{z+}]}{[L]^n}.$$

Rearranging equation (2), one obtains

$$(7)\quad \frac{[ML_n^{z+}]}{[L]^n} = K_2[M^{z+}].$$

Substitution of equation (7) into (6) then yields

$$(8)\quad [MX^{z+}\text{–X–cell}] = K_5 \cdot K_2[X\text{–cell}][M^{z+}]$$

which shows the same dependence on M^{z+} as equation (4′). For our present purposes, however, we shall continue to use equations (4) and (4′) as the simplest representation of the interaction of the metal with the cell surface. The mechanism leading to a biological effect might then involve the reaction of M^{z+} at a metabolically active site on the cell surface, resulting in a direct biological response (Pagenkopf 1983); alternatively, binding at the cell surface site might simply precede transport into the cell, the reaction with the metabolically active centre occurring intracellularly (Harrison and Morel 1983; Sunda and Huntsman 1983).

Functional groups potentially present at cell surfaces, and thus conceivably involved in metal binding, include the following: alcohol, carboxylic acid, amine, amide, hydroxamic acid, phosphate ester, thiol, imidazole (Beveridge and Murray 1980; Doyle et al. 1980; Crist et al. 1981; Nelson et al. 1981). This binding may involve individual functional groups or, more likely, a concerted action between different functional groups, e.g. chelation. Clearly there exists a potential for binding cations other than M^{z+} at these sites, notably the hydrogen ion itself, H^+. Depending upon the pK values of the functional groups involved, changes of pH might thus affect the degree of protonation of the binding sites.

$$(9)\quad H^+ + X\text{–cell} \overset{K_6}{\rightleftarrows} H^+\text{–X–cell}.$$

In such a case, the proton would play a protective role analogous to the well-documented protection afforded by the hardness cations, Ca^{2+} and Mg^{2+} (Borgmann 1983; Pagenkopf 1983), reducing the availability of the binding sites for toxic metals. An increase in the H^+ concentration might thus be expected to influence the distribution of M^{z+} among the various cellular ligands, X–cell, present at the cell surface and, in some cases, to lead to a decrease in the overall concentration of surface-bound metal.

Several additional points merit consideration. First, the reasoning developed to this point strictly applies to situations where equilibrium exists between the metal present in solution and the metal bound at the cell surface. If the reaction of M^{z+} at the cell surface (equation (4)) is *not* reversible, then the biological response will be determined not by thermodynamic equilibrium considerations but rather by the kinetics of the reaction of M^{z+} with the cellular ligand:

$$(10)\quad M^{z+} + X\text{–cell} \overset{k}{\rightarrow} M^{z+}\text{–X–cell}.$$

An increased H^+ concentration would decrease the proportion of unprotonated binding sites, X–cell, and reduce the rate of

formation of the biologically relevant M^{z+}–X–cell surface complex. Thus even under conditions of kinetic control, decreases in pH could retard the biological response. Secondly, changes in pH might also induce allosteric conformational changes at the metal-binding sites and thus alter their affinities (pK_A) for the toxic metal of interest. Finally, of importance for those metals moving passively across the cell membrane, changes in pH may affect the membrane potential. In order to regulate intracellular pH and maintain a negative potential across the membrane, protons are pumped out by the cell (Raven 1980). As the pH is lowered, there will be an increasing tendency for H^+ to enter the cell, and the proton pumps will respond to maintain the internal charge differential. Eventually, depolarization of the membrane may occur, related to the decreased pH, with consequent effects on the uptake of metal ions.

Scope

Prior to approximately 1970, the effects of pH changes on aquatic organisms, in the presence or absence of trace metals, were generally interpreted in terms of the physiological response of the organism, with little or no consideration of pH-induced chemical changes in the external medium. In recent years, however, the research pendulum has swung in the opposite direction and the effects of pH changes now tend to be interpreted in terms of speciation changes in the external medium. As already discussed, a decrease in pH may reasonably be expected to affect *both* metal speciation in solution *and* biological sensitivity at the cell surface level. These two responses to acidification are antagonistic and have the potential to cancel each other; the observed overall response at the organism level to a pH change from 7 to 4, at constant total metal concentration, may well be positive, negative, or null.

Within this framework, using in large measure previously published results, we have attempted to distinguish between changes in metal speciation occasioned by a decrease in pH from 7 to 4 and the concomitant changes in biological sensitivity over this same pH range. The specific questions addressed in this review are as follows.

(1) Does the literature provide evidence that the response to metals of aquatic biota is pH dependent?

(2) For individual metals, are there any empirically derived pH-related patterns that agree with those expected theoretically?

(3) Do producer and consumer organisms respectively differ in their response to pH–metal combinations?

(4) If low pH does change metal uptake or toxicity, does this happen at realistic pH and metal combinations, i.e. would there be a practical aspect or is it effectively of academic interest only?

Results and Discussion

pH-Induced Changes in Metal Speciation

Our attention was focused initially on those metals that exist predominantly in cationic forms and can be considered "very toxic and relatively accessible" in an environmental context (Wood 1974). Several metals were deleted from this compilation, there being few or no environmental data available (Au, Be, Bi, Pd, Pt, Sb, Te, Tl), and aluminum was added because of its known implication in the response of watersheds to acid deposition (Dickson 1978; Cronan and Schofield 1979; Johnson et al. 1981). The final list of 10 metals we considered included Ag, Al, Cd, Co, Cu, Hg, Mn, Ni, Pb, and Zn.

Possible approaches to the determination of the speciation of trace metals in natural waters can be grouped into two major categories (Florence and Batley 1980; Florence 1982): (i) thermodynamic calculations, involving the use of total concentrations ($[M]_T$, $[L]_T$) as well as pH, E_H, T, and published stability-constant values, to compute the equilibrium contribution of the various species (e.g. Jenne 1979); and (ii) experimental methods, involving the determination of the physical properties (e.g. size) and/or chemical reactivity (e.g. electrochemical lability, ion-exchange behavior) of the trace metal of interest. In the former case, provided that the assumption of equilibrium is valid, the major current limitation is the absence of reliable thermodynamic data for many of the species suspected to be present in natural waters (notably those involving natural organic matter, present as dissolved ligands or as colloïdal organic material); Nordstrom et al. (1979) have demonstrated the sensitivity of these equilibrium models to the choice of stability constants serving as input data. In addition, the ability of these simple models to represent metal interactions with multifunctional polyelectrolytes (e.g. fulvic acids) has been questioned (Perdue and Lytle 1983). The second or experimental approach is limited by the lack of experimental techniques sufficiently sensitive and selective to detect individual trace metal forms at the concentrations normally found in natural waters. Current techniques allow only the classification of trace metal forms into various operationally defined categories, according to their physical properties or chemical reactivity. Since the thermodynamic and experimental approaches are clearly complementary, both have been considered in the present study.

Equilibrium calculations

Thermodynamic calculations were used to identify which of the metals of current concern are most likely to be affected by pH changes. For the present purposes, the major ion composition of an "average surface water" was derived from data available for 10 representative lakes located in the Laurentide Park area on the Canadian Precambrian Shield north of Quebec City (Table 1). Note that these lakes are typical of the low hardness/low alkalinity waters found on the Shield in southern Quebec

TABLE 1. Bulk composition of the average lake water used for calculation of the effect of acidification on trace metal speciation (average observed concentrations for 10 lakes in the Laurentide Park area on the Canadian Shield north of Quebec City (Campbell et al. 1982)).

Constituent	Concentration (μmol/L)
Calcium	38.9
Magnesium	14.5
Potassium	5.9
Sodium	26.9
Iron	4.1
Manganese	0.9
Aluminum	5.9
Sulfate	36.4
Chloride	11.3
Fluoride	2.6
Nitrate	35.5
Phosphate	0.1
Inorganic carbon	332

TABLE 2. Effect of acidification on the calculated speciation of dissolved trace metals in a typical surface water from the Canadian Shield (no organic ligands, $p\epsilon = 12$).

Metal	Total concentration (μmol/L)	Percent aquo ion[a] $M^{z+}(H_2O)_n$ pH 7	pH 6	pH 5	pH 4	Other dissolved species (≥1%)	Solids
Ag	0.01	99	99	99	99	$AgCl$	
Al	5.89	<1*	<1*	<2*	58	AlF^{2+}, AlF_2^+, $AlOH^{2+}$	$Al(OH)_3$
Cd	0.10	99	100	100	100		
Co(II)	0.01	100	99	100	100		
Cu	0.10	37	96	100	100	$CuOH^+$, $Cu(OH)_2^0$, $CuCO_3^0$	
Hg	0.001	<1	<1	<1	<1	$HgCl^+$, $HgCl_2^0$, $HgClOH^0$, $Hg(OH)_2^0$	
Mn	0.72	<1*	50*	100	100		MnO_2
Ni	0.10	99	100	100	100		
Pb	0.005	36	91	100	100	$PbOH^+$, $PbCO_3^0$	
Zn	0.50	99	100	100	100		

[a]An asterisk indicates the presence of a precipitated solid (see last column); % = ([aquo ion] ÷ $[M]_T$) × 100.

(hardness <15 mg $CaCO_3$/L; alkalinity <120 μeq/L: Lachance et al. 1985). Trace metal concentrations were assigned either average observed values (Al, Mn) or arbitrary but representative values (Table 2, column 2); provided that ligands are present in excess (i.e. [L] ≫ [M]), the relative proportion of each metal form will be independent of [M]. The MINEQL-1 chemical equilibrium model (Westall et al. 1976) was used to calculate the theoretical speciation of each trace metal.

Simulations were performed for aerobic conditions (redox potential, $p\epsilon$, of 12) at fixed pH values of 7, 6, 5, and 4 for the simplest inorganic system (no organic ligands present; no adsorbing solid surfaces). Based on the results of these simulations (Table 2), five metals (Ag, Cd, Co, Ni, Zn) exist predominantly (>99%) as aquo ions throughout the studied pH range. For the remaining metals, however, significant changes in speciation are predicted to occur as the pH decreases from 7 to 4. In this latter class we find metals that show significant changes in physical speciation (percent dissolved metal) as the pH decreases (Al, Mn) and also metals for which the equilibrium distribution among dissolved species shifts (Al, Hg; to a lesser degree Cu, Pb). In this simple inorganic system, the pH change from 7 to 4 particularly affects those metals that undergo extensive hydrolysis and those forming strong carbonato complexes.

A second series of simulations was performed for a system containing, in addition to the above constituents, eight model organic ligands (arginine, lysine, ornithine, valine; citric, maleic, phthalic and salicylic acids). The relative proportions of each ligand were those used by Sposito in his "Mixture Model," which was designed to simulate the acid–base and metal-complexing behavior of a fulvic acid (Sposito 1981); the total ligand concentration of 22 μmol/L corresponds to a fulvic acid DOC concentration of 10 mg organic C/L. Based on the results of these simulations, only Al and Cu interact appreciably with the added ligands (Table 3). Reducing the pH from 7 to 4 decreases the degree of complexation of each of these metals, this decrease being greater in the presence of the organic ligands than in the inorganic system described above.

Experimental results

In view of the known sensitivity of chemical equilibrium models to the choice of stability constants used as input data (Nordstrom et al. 1979), it seemed advisable to compare the predictions of the preceding section with experimental data relating to natural waters. Unfortunately, such data proved to be surprisingly scarce! As indicated in Table 4, two experimental approaches have generally been used to follow shifts in metal speciation occurring in response to pH changes[1]: electrochemical determinations (potentiometric; polarographic) and size separations (dialysis). Each of these is now discussed.

TABLE 3. Effect of organic ligands on the sensitivity of Al and Cu speciation to pH changes.

Metal	Total concentration (μmol/L)	Organic ligands[a]	Degree of complexation[b] pH 7	pH 6	pH 5	pH 4
Al	5.9	No	4.46*	1.40*	0.87*	0.23
		Yes	7.43	4.45	1.58	0.38
Cu	0.1	No	0.43	0.0	0.0	0.0
		Yes	1.44	0.15	0.0	0.0

[a]Sposito "Mixture Model" (see text for details).

[b]Degree of complexation = ΔpM = log ([total dissolved metal] ÷ $[M^{z+}]$). An asterisk indicates the presence of a precipitated solid, $Al(OH)_3(s)$.

The least ambiguous results are those obtained by direct potentiometric determination of the free metal ion activity. However, few of the metals of current concern (Ag, Cd, Cu, and perhaps Pb) are amenable to direct determination with ion-selective electrodes at realistic concentrations not too far removed from environmental levels; studies carried out under such conditions, close to the analytical detection limit and in the presence of variable H^+ concentrations, are fraught with difficulty.

Engel et al. (1981) spiked a filtered organic-rich river water with Ag, Cd, or Cu (1 μmol/L) and lowered the pH from 7.5 to 5.5. The activity of the free aquo metal ion, as determined with the appropriate ion-selective electrode, increased markedly in the case of Cu; similar but much more modest increases were noted for Cd and Ag. These trends were interpreted in terms of increased competition by H^+ for the available ligands as the system was acidified (cf. equations (2) and (3)). For Cd this modest pH dependence vanished after UV-photooxidation, suggesting that the ligands involved were organic in nature; the effects of photooxidation on Ag and Cu were not reported.

[1]Shifts in metal speciation will be discussed in terms of the "degree of complexation" of the metal, ΔpM, which for the present purposes has been defined as ΔpM = log ([total dissolved metal] ÷ $[M^{z+}]$), as shown in Stumm and Morgan (1981, p. 358).

TABLE 4. Experimental evidence for pH-induced changes in the speciation of dissolved metals.

Analytical technique[a]	Metal [μmol/L]	Sample	pH range	pH dependence	Comments[b]	Reference
ise	Cd [0.9–9]	Synthetic	7.4–5.0	Low	With/without HA (7–86)	Gardiner 1974
ise	Cu, Pb [0.2–1]	Synthetic	7.0–2.0	High (pH < 6)	With HA (10)	Guy and Chakrabarti 1976a
ise	Cu [20]	Soil extract	7.6–6.3	High (pH < 7)	—	McBride and Bouldin 1984
ise	Ag, Cd, Cu [1]	River water	7.5–5.5	Cu ≫ Cd > Ag	—	Engel et al. 1981
asv	Cu, Pb, Zn [2.5]	Synthetic	9.0–5.0	Cu ~ Pb > Zn	With $NaHCO_3$ (≤0.1 mol/L)[c]	Ernst et al. 1975
asv	Cu [1]	Synthetic	8.0–2.0	High (pH < 7)	With HA (11)	Guy et al. 1975
asv	Cu, Pb [0.2–1]	Synthetic	8.0–2.0	High (pH < 6)	With HA (10)	Guy and Chakrabarti 1976a
asv	Cd, Cu, Pb [0.05]	Synthetic	8.0–6.0	Cu > Pb > Cd	With HA (5–20)	O'Shea and Mancy 1978
asv	Cd, Cu, Pb [0.5–1.6]	Synthetic	7.0–4.5	Cu > Pb > Cd	With NaOAc (1 mol/L)	Schonberger and Pickering 1980
asv	Cd, Cu, Pb, Zn [2–6]	Spiked lake water	7.0–4.7	Cu, Pb > Cd > Zn	With NaOAc (0.1 mol/L); ultrafiltered (5 nm) before acidification	Kinsey 1975
Dialysis	Cd, Cu, Mn, Pb, Zn [25–90]	Synthetic	8.0–3.0	Cu, Pb > Cd, Zn, Mn	With HA (20)	Guy and Chakrabarti 1976b
Dialysis	Cu [?]	River water	8.0–2.0	High (pH < 5)	Filtered before acidification?	Guy and Chakrabarti 1976b

[a]Analytical techniques: ise = ion selective electrode; asv = anodic stripping voltammetry.
[b]Code: HA = humic acid; OAc = acetate. Humic acid (HA) concentrations given in parentheses (mg/L).
[c]Formation "insoluble species" at high pH.

A marked pH dependence of ΔpCu, as determined by potentiometric measurements, has also been reported for a soil solution extracted from a contaminated soil (McBride and Bouldin 1984) and for synthetic solutions containing humic acid (Guy and Chakrabarti 1976a). These latter workers observed that the speciation of Pb in similar synthetic solutions was also very sensitive to pH changes in the critical region 7–4. The relative insensitivity of Cd speciation to acidification, as reported by Engel et al. (1981), is consistent with the findings of Gardiner (1974) who concluded that ΔpCd showed little or no pH dependence in synthetic solutions.

Polarographic techniques have also been used to follow shifts in metal speciation occasioned by changes in pH, both in synthetic solutions (Ernst et al. 1975; Guy et al. 1975; O'Shea and Mancy 1978; Schonberger and Pickering 1980) and in natural water samples (Guy et al. 1975; Kinsey 1975). As indicated in Table 4, increases in the concentration of "labile" metal after sample acidification have been reported for several of the metals of current concern; generally the pH dependence decreases in the order Cu, Pb ≫ Cd, Zn. However, changes in pH may alter the polarographic reduction kinetics of the various labile metal species (Shuman and Michael 1978) and also influence the adsorption of dissolved organic matter on the working electrode (Brezonik et al. 1976). Thus the electrode response itself is affected by acidification of the sample, and changes in the analytical signal (i_p; E_p) cannot be ascribed solely to pH-induced changes in metal speciation in the bulk solution.

Changes in the size distribution of metal species as a function of pH have been studied by Guy and Chakrabarti (1976a, 1976b), who used equilibrium dialysis to differentiate between high molecular weight metal species (colloïdal and pseudo-colloïdal forms; metals associated with fulvic or humic acids) and smaller dialyzable forms (simple inorganic species; small organic complexes). Synthetic solutions containing humic acid and individual metals (Cd, Cu, Mn, Pb, Zn) were adjusted to different pH values in the range 8–3 and then dialyzed (nominal molecular weight cutoff ~14 000). In all cases the percent dialyzable metal increased as the pH decreased, a trend that was interpreted by the authors as indicating progressive decomplexation on acidification; possible effects of pH on membrane permeability itself were not explicitly considered (cf. Staub et al. 1984). The dialysis behavior of Cu and Pb showed a more marked sensitivity to pH changes than did that of Cd, Mn, or Zn:

	% dialyzable	
	pH 7	pH 4
Cu, Pb	0–5	60–80
Cd, Mn, Zn	40–60	90–100

For Cu these apparent shifts in size distribution also occurred in a river water sample, but at a lower pH than in the synthetic samples; the percent dialyzable Cu remained very low (<10%) until pH ~ 5, and then increased markedly.

Without exception the results described above were obtained in the laboratory, either with synthetic solutions or with surface water samples. To our knowledge, the only field study designed to evaluate pH-induced changes in metal speciation is that carried out in limnocorrals in the Experimental Lakes Area (Jackson et al. 1980; Schindler et al. 1980). Lowering the pH within the enclosures from 6.7 to 5.7 to 5.1 affected both the physical speciation (ratio of dissolved metal to particulate metal) and the reactivity (loss rate from the water column) of some but not all of the added radioisotopes (^{60}Co, ^{203}Hg, ^{54}Mn, ^{65}Zn). With reference to this latter parameter, which reflects removal of the radioisotope either by sedimentation or by direct adsorption on bottom sediments, only for Mn and Zn did the loss rates from the water column decrease significantly when the pH was lowered from 6.7 to 5.1.

Summary — metal speciation in solution

For those metals for which changes in speciation as a function of pH have been studied experimentally, reasonable agreement with their predicted sensitivity to pH changes is observed:

Metal	Predicted	Observed
Ag	Low	Low
Al	High	?
Cd	Low	Low
Co	Low	?
Cu	High	High
Hg	High	?
Mn	Low	Low
Ni	Low	?
Pb	Moderate	High
Zn	Low	Low

In effect, for the four metals (Al, Cu, Hg, Pb) predicted to manifest significant speciation changes in the range pH 7–4, confirmatory experimental data are available for two (Cu, Pb). Similarly, in the six remaining cases predicted to show little sensitivity to pH changes in this range, supporting experimental evidence exists for four metals (Ag, Cd, Mn, Zn). These observations remain essentially qualitative, however, and there is a need for quantitative data relative to the pH sensitivity of metal interactions with natural organic ligands.

pH-Induced Changes in Metal Adsorption at Biological Surfaces

Much information about the influence of pH changes on metal adsorption at *abiotic* surfaces exists in the scientific literature (e.g. metal oxides and oxyhydroxides (Anderson and Rubin 1981; Stumm and Morgan 1981); humic materials (Kerndorff and Schnitzer 1980)). In contrast, relatively few data exist regarding the pH dependence of metal adsorption at biological surfaces; the literature search undertaken for the present review turned up some 16 such studies (Table 5), the majority having been performed on algae (13 of 16). In these studies the results are generally reported either as metal concentration factors (CF = $[M]_a/[M]_s$, where $[M]_a$ = concentration of the metal associated with the alga and $[M]_s$ = metal concentration in solution) at different pH values (Table 5: cases 4–9) or as metal adsorption isotherms as a function of pH (cases 1–3, 11–15). As the experimental procedures employed and the methods used for data reduction and analysis vary greatly, it has unfortunately proved difficult to compare the results on a quantitative basis. Of even greater consequence, however, is the fact that researchers in this area have paid surprisingly little or no attention to metal speciation in the extracellular solution. Not only have possible interactions between the metal and other constituents of the incubation medium often been ignored, but in addition the pH of this medium has been raised or lowered with little or no consideration of the possible effects of this change on metal speciation. Note also that many of the studies were performed in dilute, unbuffered solutions; under these conditions the pH close to a charged surface can be markedly different from that in the bulk solution (Stumm and Morgan 1981, p. 621). In such studies there thus exists some ambiguity as to the effective pH in the immediate vicinity of the biological surface.

To uncover relatively unambiguous H^+/M^{z+} competition effects at the cell–medium interface, we formulated a number of "selection criteria" to be applied to the various studies listed in Table 5. To be retained for further consideration, studies had to satisfy most if not all of the following criteria:

(1) studied metals should be one or more of those identified earlier as least likely to change speciation in solution over the pH range of interest (i.e. Ag, Cd, Co, Mn, Ni, or Zn);

(2) a defined inorganic incubation medium, or barring that, a defined organic medium containing only weakly complexing buffers (to minimize metal speciation changes in solution as the pH is changed);

(3) total metal concentrations low enough to be environmentally relevant (<5 μmol/L);

(4) short incubation times with the added metals (to minimize metal incorporation into the test organism; surface-bound metal should predominate).

Only 10 of the original 16 studies satisfied these rather stringent criteria; three metals (Cd, Mn, Zn) and seven organisms (six algae, one bacterium) are represented (Table 6).

All seven studies performed in *inorganic* media involved living algae. Four of these investigations were carried out by Starý and co-workers, who incubated two green algae, *Chlorella kessleri* and *Scenedesmus obliquus*, with a variety of radioisotopes over a wide range of pH values (Tables 5 and 6, cases 4–7). The two algae exhibited virtually identical behavior. At a given pH, the concentration factors for each of the metals considered here were of the same order of magnitude (~10^4 at pH 7) but decreased slightly in the order Mn > Zn > Cd. The concentration factors attained their maximum values at low metal concentrations (i.e. at low surface coverage) and at the high end of the studied pH range. The pH values indicated in Table 6 correspond to the threshold below which competition between H^+ and M^{z+} was noted. Responses of the CF to decreases in pH were similar for all three metals; below the threshold pH values, plots of log CF as a function of pH were linear with slopes close to unity, increasing slightly in the order Mn (0.75) < Cd (0.81; 0.86) < Zn (0.81; 0.98).

TABLE 5. Studies of pH-induced changes in metal adsorption on cell surfaces.

Organism	Metal [μmol/L]	pH range	Time (h)	Incubation medium	Reference
1. *Bacillus subtilis* (cell walls)	Mn [?]	5.2–2.0	48	Acetate	Doyle et al. 1980
2. Mixed bacterial culture	Cd, Cu, Zn [10–100]	10–4	4	Complex organic	Nelson et al. 1981
3. Mixed bacterial culture	Cd, Cu, Ni, Zn [10 – 50 000]	8–5	24	Complex organic	Gould and Genetelli 1978a, 1978b, 1984
4. *Chlorella kessleri*	Cd, Cr, Hg, Zn [1]	10.5–4	3	Inorganic unbuffered	Starý and Kratzer 1982
5. *Chlorella kessleri*	Co, Cr, Hg, Mn, Zn [0.1–1]	9–4	3	Inorganic unbuffered	Starý et al. 1983b
6. *Scenedesmus obliquus*	Ag, Cd, Co, Cu, Mn, Pb [0.1–1]	9–4	3	Inorganic unbuffered	Starý et al. 1983b
7. *Scenedesmus obliquus*	Cd, Hg, Zn [0.5–3]	9–4	1 3	Inorganic unbuffered	Starý et al. 1983a
8. *Scenedesmus* sp. (2) *Synechococcus* sp. (2) *Oscillatoria* sp. *Chlamydomonas* sp. *Euglena* sp.	Ni [0.3]	9–4	6	Various buffers + culture medium	Wang and Wood 1984
9. *Navicula pelliculosa* *Mougeotia* sp. *Scenedesmus obliquus* *Spirogyra* sp. *Ulothrix fimbrinata* *Zygnema* sp. *Chlamydomonas* sp. *Chlorella pyrenoidosa* *Gleotrichia* sp. *Oscillatoria* sp. *Schizothrix calcicola* *Nostoc* spp.	Cd, Hg, Pb [0.2–3]	10–4	3	Various buffers + culture medium	Hassett et al. 1981
10. *Scenedesmus acuminatus*	Cu [2]	6.8–3.8	0.03	Inorganic phosphate buffer (0.5 mmol/L)	Mierle and Stokes 1976
11. *Chlorella regularis*	Cd [?]	7–3	1	Inorganic unbuffered	Sakaguchi et al. 1979
12. *Chlorella regularis*	Mn [36]	7–3	1	Inorganic unbuffered	Nakajima et al. 1979
13. *Ulothrix* sp. (cell walls)	Cu, Pb, Zn [100–1000]	6.2–3.8	24	Complex organic	Ferguson and Bubela 1974
14. *Vaucheria* sp. (cell walls)	Zn [10 000]	7–4	?	Tartrate (5×10^{-2} mol/L)	Crist et al. 1981
15. *Chroococcus paris*	Cd, Cu, Zn [90–160]	7–4	0.25	Inorganic unbuffered	Les and Walker 1984
16. *Chlamydomonas variabilis*	Zn [1–3]	7–5	0.15	Acetate or HEPES buffers + EDTA	Harrison et al. 1986

Rather similar studies were carried out with another green alga, *Chlorella regularis* (Tables 5 and 6, cases 11, 12). The algae were harvested during the log growth phase, washed, and resuspended in unbuffered inorganic solutions containing the appropriate metal (Mn(II) or Cd) as its chloride salt; after adjustment of the pH by addition of HCl or NaOH, the algae were incubated with the metal for 1 h, collected on filters, and analyzed. Results are reported not as CF but rather as metal concentrations associated with the algae (micrograms metal per gram cell dry weight). Short-term extractions of the harvested

TABLE 6. Evidence of H^+/metal ion competition at biological interfaces.

Study No.[a]	Organism	Metal	pH-sensitive parameter[b]	pH threshold for competition	Remarks
			Inorganic media		
4, 5	*C. kessleri*	Mn	CF	8	Physiological state of algae not specified
		Zn		8	
		Cd		8.5	
6, 7	*S. obliquus*	Mn	CF	8	Physiological state of algae not specified
		Zn		8	
				8	
11, 12	*C. regularis*	Mn	$[M]_a$	5	[Mn] high
		Cd		7	[Cd] not specified
15	*C. paris*	Zn	$[M]_a$	7	[Zn] high
		Cd		7	[Cd] high
			Defined organic media (weakly complexing)		
1	*B. subtilis* (walls)	Mn	$[M]_a$	4	[Mn] not specified
14	*Vaucheria* sp. (walls)	Zn	$[M]_a$	6	[Zn] and [tartrate] both very high
16	*C. variabilis*	Zn	$[M]_a$	—	EDTA-extractable Zn ~5× greater at pH 7 than pH 5

[a]Study No. refers to the compilation in Table 5.
[b]Measured parameters: CF = concentration factor = μg M/g algae ÷ μg M/cm^3 medium; $[M]_a$ = metal concentration associated with cell = μg M/g algae or bacteria.

algae with 0.1 mol EDTA/L released ~70% of the $[Mn]_a$ and ~90% of the $[Cd]_a$, suggesting that most of the metal associated with the algae after an incubation time of 1 h was indeed surface-bound (cf. Bates et al. 1982). Binding of Cd proved to be much more sensitive to pH changes than did that of Mn; in the former case the amount of Cd associated with the cells decreased linearly from pH 7 to 4 (~95% reduction) whereas for Mn no effect of increasing H^+ concentration was noted until the pH of the solution dropped below pH ~ 5 (Table 6).

The final study among those performed in an inorganic medium is that of Les and Walker (1984), who studied the adsorption of Cd and Zn on *Chroococcus paris*, a unicellular sheath forming blue-green alga (Tables 5 and 6, case 15). Their experimental procedure was very similar to that of Nakajima and Sakaguchi described earlier, except that total metal concentrations were considerably higher. For both metals, plots of $[M]_a$ as a function of pH showed slightly decreased metal binding as the pH was lowered from pH 7 to 4 (~25–30% reduction).

Of the three studies carried out in defined *organic* media, two involve cell wall preparations rather than living organisms (Tables 5 and 6, cases 1, 14). In case 1, Doyle et al. (1980) reported the pH dependence of the adsorption of Mn on cell walls isolated from the gram-positive bacterium *Bacillus subtilis*, a much studied microorganism for which the binding of metals to the wall has been examined in considerable detail (Beveridge and Murray 1976, 1980; Matthews et al. 1979; Beveridge and Jack 1982). The cell wall preparation was washed, suspended in an acetate buffer, placed in a dialysis bag, and equilibrated for 48 h with Mn^{2+} at pH values ranging from 5.2 to 2. The adsorption data were analyzed by means of Scatchard plots ($[Mn]_a/[Mn]_s$ vs. $[Mn]_a$), which yield estimates of both the number of binding sites (n = micromoles Mn bound per milligram cell wall) and the conditional association constant (K_a). When H^+ concentration was increased, the affinity of the walls for Mn decreased, but the number of metal binding sites remained constant, suggesting that Mn^{2+} and H^+ compete for the same sites. Note, however, that this competition became effective only at pH values <4.3. Such behavior is consistent with the suggestion, derived from experiments involving the chemical modification of cell wall functional groups, that the metal ion binding capacity of these walls is mediated by the available carboxylate groups in the wall fabric (Matthews et al. 1979; Beveridge and Murray 1980; Beveridge and Jack 1982).

The second study in this group was performed by Crist et al. (1981), who isolated the cell wall fraction from a natural population of *Vaucheria* sp., a yellow-green filamentous alga, and studied the pH dependence of Zn adsorption on the fragments (Tables 5 and 6, case 14). Above pH 6 the binding of Zn by the cell wall was unaffected by changes in H^+ concentration, but in the pH interval 6–5 the amount of Zn associated with the cell wall decreased by ~75%; a further ~15% reduction occurred from pH 5 to 4. Over this same pH range, as H^+ concentration increased by a factor of 100, $[Zn^{2+}]$ also increased slightly (2.2 μmol/L at pH 6; 3.4 μmol/L at pH 4); the reduction in Zn binding at low pH is most readily ascribed to increased competition by H^+ for binding sites on the cell wall surface.

Similar results were obtained in experiments with the green alga *Chlamydomonas variabilis* grown in batch culture in a defined low-Zn medium (Tables 5 and 6, case 16 (Harrison et al. 1986)). Intact cells were harvested at different stages of the growth cycle (early log phase, exponential growth, stationary phase), resuspended in a buffered medium at pH 7.0 or 5.0, and incubated for a short period (10–20 min) with radioactive ^{65}Zn. Zinc speciation in the incubation media could be calculated at

each pH value. The labelled cells were collected and the surface-bound Zn was removed by extraction with EDTA (Bates et al. 1982; cf. cases 11 and 12). At constant Zn^{2+} concentration, levels of adsorbed Zn were up to 5 times higher at pH 7.0 than at pH 5.0; this pH effect was much greater for cells harvested early in the growth cycle than for those collected during the stationary phase.

Summary — metal adsorption at biological surfaces

Unambiguous data regarding the pH sensitivity of metal interactions at biological surfaces are not readily available; in the relatively few reports to be found in the literature, the pH of the external medium has often been varied experimentally with no consideration of the possible effects of this change on the speciation of the adsorbate in solution. Nevertheless, for some of the metals predicted to change speciation only slightly over the pH range 7–4 (viz. Cd, Mn, Zn), clear evidence does exist for competition between H^+ and the aquo metal ion for binding sites on algal and bacterial cell surfaces. This competition appears to become effective at environmentally relevant pH values (e.g. pH > 5; see Table 6).

pH-Induced Changes in Metal Toxicity

Information on the response of aquatic organisms to low pH and/or elevated metal concentrations comes from a number of sources or subdisciplines of biology, limnology, and toxicology. However, much of the published material on metal–pH–organism interactions is difficult to interpret in terms of the respective effects of pH on chemical speciation in the environment and on metal uptake by organisms.

One type of information results from concerns over effects of acidification on biota, and utilizes field surveys and field experiments across spatial gradients of $[H^+]$. These studies have consistently revealed the absence of certain plant and animal species at the lower end of the pH range 7–4. For example, very few blue-green algal species are found in the plankton of acidic lakes (Yan and Stokes 1978; NRCC 1981); the lower pH limit for a number of fish species is well documented (NRCC 1981); certain diatoms have such consistent pH preferences that diatoms are used as indicators of acidification, i.e. to infer pH (Davis et al. 1983). Such circumstantial evidence of pH tolerance or preference does collectively suggest that the number of species that can live at pH values <5.5 is smaller than the number found in circumneutral waters. Because of the correlation between pH and the total concentration of metals such as Al, Mn, and Zn in surface waters (Wright and Snekvic 1978; Dickson 1980; Bailey and Stokes 1984) it can be assumed that organisms that tolerate low pH also tolerate elevated concentrations of metals in their environment. In the absence of detailed determinations of metal speciation in these field situations, however, the respective effects on biota of H^+ and metals cannot be separated.

The second type of study involves experimental manipulation of pH and metals in test solutions, in laboratory or (less frequently) in field experiments. Even in such studies, depending upon the researchers' objectives, information about the external chemical environment is often lacking. Algae are often raised in an undefined medium with "soil water" or lake water as a major component; in many instances this is necessitated by the problems associated with developing a chemically defined medium in which the organism of interest will grow. Heterotrophic microorganisms require an organic carbon source and frequently other organic nutrients; the use of such ingredients as yeast extract and proteose peptone, while apparently permitting good growth, makes it difficult, if not impossible, to determine the chemical forms of metals. Such nondefined media also have the disadvantage that successive batches of media may not be exactly the same. For consumers, there is an added complication because of the potential for accumulation via the ingested food as well as by direct uptake of metals from water. All laboratory studies are targets for criticism concerning their relationship to the "real world," but for the purpose of the questions addressed in this review, controlled laboratory studies are extremely relevant.

Zinc

Zinc is a ubiquitous contaminant, and even in the absence of point sources the concentration of Zn in surface waters increases as pH decreases (NRCC 1981). As discussed earlier, the chemical speciation of Zn varies little over the pH range 7–4. Therefore, in a defined system with no abiotic solid phase, any measured pH–Zn interaction is expected to be governed by the response of the organism. Recent Zn–pH studies for algae, fish, and crayfish are summarized in Table 7.

Studies on the growth of the filamentous green alga *Hormidium rivulare* were carried out in defined medium (modified Chu No. 10), over a pH range of 7.0–2.5 (Hargreaves and Whitton 1976). These researchers used isolates of the alga from four sites, differing in Zn content, pH, and Cu content; it is important to note that the authors considered *H. rivulare* to be acid tolerant, regardless of the pH of the site of origin. Toxicity tests with the acid stream isolate showed that Zn was least toxic over the pH range 3.5–4.0, which was optimal for growth. Similarly, for the blue-green alga *Anacystis nidulans* (Shehata and Whitton 1982) a Zn-tolerant strain showed a decreased toxic response when the pH was lowered from 8.0 to 6.5, but a Zn-sensitive strain showed an increased toxic response when the pH was similarly lowered. For another green alga, *Stigeoclonium tenue*, there was a much less clear pH effect, but for a Zn-sensitive population, lowering the pH from 7.7 to 7.1 brought about a slight increase in Zn toxicity (Harding and Whitton 1977). Rai et al. (1981) demonstrated an increased toxicity of Zn to *Chlorella vulgaris* as the pH decreased, and attributed this observation to increased Zn "availability."

A recent reference to studies with aquatic phycomycetes *Achlya* and *Saprolegnia* (Babich and Stotzky 1983) describes a decrease in Zn toxicity as the pH was lowered from 7.5 to 5.5; no change in toxicity occurred over the range 9.5–7.5. This latter observation is surprising, in that very high Zn concentrations were employed (Table 7) and Zn precipitation might have been anticipated at the pH values >7.5. This solubility property was considered by Sprague (1964) as the explanation for the increased "resistance time" (decreased toxicity) that occurred in rainbow trout (*Salmo gairdneri*) when the pH was raised from 7.9 to 9.3. No lower pH values were tested in this study, but more recently in the same laboratories Bradley and Sprague (1985) examined the response of *S. gairdneri* to Zn, in relation to water pH and hardness. For both low (31 mg $CaCO_3$/L) and high (386 mg $CaCO_3$/L) hardness, Zn toxicity was less at pH 5.5 than at pH 7. Two competing mechanisms appear to operate: at successively higher pH levels, dissolved Zn becomes increasingly toxic, but at high pH (7–9.0) it is increasingly replaced by particulate Zn, which is of very low toxicity to fish. Similar results were obtained in a recent fish toxicity study on small

TABLE 7. Zinc uptake and toxicity in various aquatic organisms as related to pH.

Organism	[Zn] (μmol/L)	Form[a]	pH	Medium	Observed effect of ΔpH	Reference
Chlorella vulgaris	380	T	10–4	CHU-10	Lower pH, more toxic (growth)	Rai et al. 1981
Hormidium rivulare	<470	T	7.0–2.5	Modified CHU-10 (defined)	Lower pH, less toxic (growth)	Hargreaves and Whitton 1976
Stigeoclonium tenue, Zn sensitive	<12	F	7.6–6.1	Modified CHU-10 (defined)	Lower pH (7.6→7.1), slightly more toxic (growth); no effect pH 7.1→6.6→6.1	Harding and Whitton 1977
Stigeoclonium tenue, Zn tolerant	<110	F	7.6–6.1	Modified CHU-10 (defined)	Lower pH (7.6→7.1→6.6), more toxic (growth); no effect pH 6.6→6.1	Harding and Whitton 1977
Anacystis nidulans	<15	T	8.0–6.5	Modified Kratz & Meyers (defined)	Lower pH, less toxic (growth) for Zn-tolerant strain; more toxic for Zn-sensitive strain	Shehata and Whitton 1982
Chlamydomonas variabilis	3	F A	7, 5	Inorganic + acetate + HEPES + EDTA (defined)	Lower pH, less Zn uptake	Harrison et al. 1986
Saprolegnia sp., *Achyla* sp.	<5	T	9.5–5.5	Complex organic; solid	Lower pH (7.5→5.5), less toxic (growth); no effect pH 9.5→7.5	Babich and Stotzky 1983
Orconectes virilis		T	6.7, 5.1	Lake water	Lower pH, less Zn in hepatopancreas than at pH 6.7	Chang et al. 1983
Salmo gairdneri	<161	F A	9.0–5.5	Hard or soft water	Lower pH, less toxic (at two hardness levels) (LC50) and slower Zn uptake in gills	Bradley 1984; Bradley and Sprague 1985
Salmo gairdneri	<14	T	7.0–4.7	Diluted well water	Lower pH, less toxic (LC50)	Cusimano et al. 1986

[a]Results are expressed on the basis of the concentrations of total metal (T), filterable metal (F), or free aquo ion (A).

steelhead (*Salmo gairdneri*) (Cusimano et al. 1986); the 96-h LC50 values for Zn in soft water increased dramatically (10 times) as the pH was decreased from 7.0 to 5.7 to 4.7.

Finally, in a field enclosure study, Chang et al. (1983) examined Zn uptake by the crayfish *Orconectes virilis*. The pH of the enclosure was lowered from the ambient value of approximately 6.7 to pH 5.3; for the animals held at pH 5.3, the hepatopancreas contained less Zn than for those held at pH 6.7. This is of particular interest because the hepatopancreas represents a secondary site of metal accumulation resulting from transport of the metal within the animal. Other tissues of the crayfish did not show pH-related differences in Zn uptake. Zinc did accumulate in gills but there was no pH effect.

Copper

Copper provides a contrast to Zn, since it is also a required trace element and is known to accumulate in living tissues, but as mentioned earlier its chemical speciation is strongly pH dependent. Therefore, it is anticipated that more complex effects may be seen when the effect of pH on Cu uptake is examined. Some recent literature relating to Cu uptake and toxicity as a function of pH is summarized in Table 8.

Some early work on Cu–pH–algae interactions was reported by Steemann Nielsen et al. (1969) and Steemann Nielsen and Kamp-Nielsen (1970). The green alga *Chlorella pyrenoidosa* was exposed to Cu in a defined medium over the pH range 8–5. Both photosynthesis and growth were more affected by Cu at pH 8 than at pH 5. Mierle and Stokes (1976) measured Cu uptake from an inorganic medium (phosphate buffer ~0.5 mmol/L) over a period of hours, and showed that the green alga *Scenedesmus acuminatus* accumulated much more intracellular Cu at pH 6.8 than at pH 5.6 or 4.8. Although this study did not consider Cu toxicity, it did deal with intracellular accumulation of Cu and is included here since subsequent work (Mierle 1982) demonstrated that this intracellular Cu was toxic. Peterson et al. (1984) measured the effect of pH on the sublethal toxicity of Cu to *Scenedesmus quadricauda*, as determined by the effect of metal additions on phosphate uptake. Normalizing for pH effects on Cu speciation, these researchers found a 76-fold decrease in Cu toxicity over the pH range 6.5–5.0, which they interpreted as resulting from competition between H^+ and the cupric ion, Cu^{2+}. Their elegant experiments provide some of the most useful data in the context of our review, but they point out that in more complex natural waters "this simple relationship may be hidden."

For several fungi, e.g. *Fusarium lycopersici* (Scott 1926, 1929), *Scytalidium* sp. (Starkey 1973), and *Penicillium nigricans* (Singh 1977), the same effect of pH on Cu was shown,

TABLE 8. Copper uptake and toxicity in various aquatic organisms as related to pH.

Organism	[Cu] (μmol/L)	Form[a]	pH	Medium	Observed effect of ΔpH	Reference
Chlorella pyrenoidosa	<2	T	8, 5	Inorganic + citrate (defined)	Lower pH, less toxic (growth)	Steemann Nielsen and Kamp-Nielsen 1970
Chlorella pyrenoidosa	<2	T	8, 5	Inorganic + citrate (defined)	Lower pH, less toxic (photosynthesis), less Cu uptake	Steemann Nielsen et al. 1969
Hormidium rivulare	<80	T	6.0–2.8	Modified CHU-10 (defined)	Lower pH, less toxic (growth)	Hargreaves and Whitton 1976
Scenedesmus acuminatus	1.6	T	6.8–3.8	Inorganic (defined)	At pH ≤ 5.8, less Cu uptake than at pH 6.8	Mierle and Stokes 1976
Scenedesmus quadricauda	<25	T A	8.5–5.0	Inorganic (defined)	At pH < 6.5, Cu^{2+} less toxic (P uptake)	Peterson et al. 1984
Penicillium nigricans	$<4 \times 10^4$	T	7.2–2.8	Complex organic	Lower pH, less toxic (growth)	Singh 1977
Scytalidium sp.	$<10^4$	T	6.8–2.0	Inorganic + glucose (defined)	Lower pH, less toxic (growth)	Starkey 1973
Fusarium lycopersici	<500	T	7.5–3.0	Inorganic (defined)	Lower pH, less toxic (growth)	Scott 1929
Daphnia magna	<5	F A	8.0–7.4	Lake water	Lower pH, Cu^{2+} less toxic (mortality rate, reciprocal survival time)	Andrew et al. 1977; Magnuson et al. 1979
Daphnia magna	<1.4	A	8.4–5.9	Inorganic (defined)	Lower pH, Cu^{2+} less toxic (LC50)	Borgmann 1983
Salmo gairdneri	<3.1	T A	8–6	Well water	Lower pH, more toxic (LC20; critical swimming velocity)	Waiwood and Beamish 1978
Salmo gairdneri	<8	F	9–5	Well water	Lower pH, less toxic (LC50)	Howarth and Sprague 1978
Salmo gairdneri	<2	T	5.4–4.3	Artificial freshwater (defined)	At pH < 4.7, antagonism between Cu and H^+; at pH 5.4, synergism (ILC50)	Miller and Mackay 1980
Salmo gairdneri	<1	T	7.0–4.7	Diluted well water	Lower pH, less toxic (LC50)	Cusimano et al. 1986

[a]Results are expressed on the basis of the concentrations of total metal (T), filterable metal (F), or free aquo ion (A).

namely that at low pH the fungi tolerated Cu better than at high pH. It should be noted that these latter two are acidophilic fungi and the improved Cu tolerance at low pH could have been an apparent rather than a real effect. Note also that total Cu concentrations in these studies were particularly high. Although not discussed by the various authors, the effect of Cu release from organic compounds in the fungal growth media with decreasing pH would tend to reverse the observed effect, so that in fact if the data were expressed in terms of the free copper ion activity, the reduced toxicity at low pH would probably be even more striking.

In a study of the effects of Cu on *Daphnia magna*, Andrew et al. (1977) demonstrated that a slight increase in H^+ concentration (pH 8.0–7.6) led to a marked decrease in the toxicity of the free Cu^{2+}. Similar results were reported by Borgmann (1983) for experiments with the same species conducted over a somewhat wider pH range (8.4–5.9).

Several studies have been made on Cu–pH interactions as they relate to fish toxicity. Howarth and Sprague (1978) measured lethal Cu concentrations (LC50) for rainbow trout over the pH range 7–5, with varying water hardness, and concluded that Cu^{2+} and $Cu(OH)_n^{(2-n)+}$ were both more toxic at the higher pH. Miller and Mackay (1980), also using rainbow trout, found an "entirely unexpected" antagonistic interaction of Cu and H^+ below pH 5.4, unexpected because they had anticipated that higher "free" Cu levels at low pH would enhance toxicity. They proposed that at low pH, mucus secretion (on the gills?) was stimulated, and that the mucus chelated Cu even at pH 3.5. They did not, however, give data on Cu uptake or on Cu bound with mucus. Copper toxicity to steelhead fry was studied by Cusimano et al. (1986) in continuous-flow softwater systems, and a very clear increase in LC50 values at lower pH was demonstrated, for 96- and 168-h tests. The authors explained their results in terms of a competition between H^+ and the added Cu for metal binding sites at the gill surface. They considered and rejected the hypothesis that the lower pH ameliorates metal toxicity by stimulating the production of mucus (which could complex the Cu), because their flow-through system "would

TABLE 9. Cadmium uptake and toxicity in various aquatic organisms as related to pH.

Organism	[Cd] (μmol/L)	Form[a]	pH	Medium	Observed effect of ΔpH	Reference
Chlorella pyrenoidosa	<9	T	8, 7	Inorganic + citrate + Tris (defined)	Lower pH, more toxic (growth) and more Cd uptake	Hart and Scaife 1977
Chlorella pyrenoidosa	<9	T	8.3–6.6	Inorganic (defined)	Lower pH, less toxic (growth) and less Cd uptake	Gipps and Coller 1980
Coelastrum proboscideum	<0.6	T	6.2–4.3	Inorganic (defined)	Lower pH, less toxic (growth)	Müller and Payer 1979
Scenedesmus quadricauda	<5000	T A	8.5–5.5	Inorganic (defined)	At pH < 7.5, Cd^{2+} less toxic (P uptake)	Peterson et al. 1984
Aspergillus niger	3.8	T	7.2–5.1	Soil	Lower pH, less toxic (growth)	Babich and Stotzky 1977
Aspergillus fischeri						
Salmo gairdneri	<0.3	T	7.0–4.7	Diluted well water	Lower pH, less toxic (LC50)	Cusimano et al. 1986

[a]Results are expressed on the basis of the concentrations of total metal (T) or free aquo ion (A).

TABLE 10. Aluminum uptake and toxicity in various aquatic organisms as related to pH.

Organism	[Al] (μmol/L)	Form[a]	pH	Medium	Observed effect of ΔpH	Reference
Chlorella pyrenoidosa	<3	T A	7.1–4.9	Inorganic (defined)	Maximum growth inhibition pH 5.8–6.2; less toxic at lower or higher pH	Helliwell et al. 1983
Catostomus commersoni (eggs)	<19	T	5.6–4.2	Dechlorinated tap water	At pH < 4.8, antagonism between Al and H^+ (% survival; % swim-up; growth)	Baker and Schofield 1982
Salvelinus fontinalis (eggs)	<19	T	5.6–4.2	Dechlorinated tap water	At pH < 4.8, antagonism between Al and H^+ (% survival; % swim-up; growth)	Baker and Schofield 1982
Salmo gairdneri	<2.8	T	6.1–4.0	Dechlorinated tap water	Maximum toxicity pH 6.1 and 4.5; less toxic at pH 5.0 (blood chemistry; ventilation parameters; activity)	Neville 1985
Bufo americanus (eggs)	<7.4	T A	5.8–4.1	Inorganic (defined)	No effect (hatching success)	Clark and LaZerte 1985
Rana sylvatica (eggs)						

[a]Results are expressed on the basis of the concentrations of total metal (T) or free aquo ion (A).

seem to exclude the possibility of gross amounts of mucus in the water matrix chelating the metal. ..."

Cadmium

In contrast with Cu and Zn, Cd is a nonessential element. It has a rather high inherent toxicity to aquatic biota (Kaiser 1980; Marshall et al. 1981) and its solution chemistry in freshwaters resembles that of Zn in that the free cadmium aquo ion, Cd^{2+}, is the dominant form over the entire pH range 7–4. The few available data from toxicological experiments on algae and fish indicate that H^+ ameliorates Cd toxicity (Gipps and Coller 1980; Peterson et al. 1984; Cusimano et al. 1986). However, for most of the work on heterotrophic microorganisms, the trends are much less clear cut, possibly because of the ubiquitous chelators present in the media used to grow the microorganisms. Studies are summarized in Table 9.

Other metals

Far fewer suitable studies are available for the other metals of concern. Pertinent results are summarized for Al (Table 10), Hg (Table 11), and Pb (Table 12).

Summary – metal uptake and toxicity

In the majority of the studies cited, the toxicity of Cd, Cu, and Zn decreased as the pH decreased. For Cd and Zn, these results are consistent with the predictions in earlier sections; for Cu,

TABLE 11. Mercury uptake and toxicity in various aquatic organisms as related to pH.

Organism	[Hg] (μmol/L)	Form[a]	pH	Medium	Observed effect of ΔpH	Reference
Chlorella vulgaris	2 0.004	T; inorganic T; CH_3HgX	10–4	CHU-10	Lower pH, more toxic (growth)	Rai et al. 1981
Ankistrodesmus sp.	<50	T; inorganic	7, 5	Inorganic + EDTA + phosphate (0.24 mol/L) (defined)	Lower pH, more toxic (growth)	Baker et al. 1983
Oocystis marsonii	0.5	T; CH_3HgX	7.0, 5.5	Inorganic (defined)	Lower pH, less Hg uptake	Richman and Stokes 1985
Fusarium lycopersici	9.2	T; inorganic	7.5–3	Inorganic (defined)	Lower pH, less toxic (growth)	Scott 1929
Orconectes virilis	Radiotracer	T; inorganic	6.7–5	Lake water	At pH < 6.7, lower bioconcentration factor for 7 separate tissues	Chang et al. 1983

[a]Results are expressed on the basis of the concentrations of total metal (T).

TABLE 12. Lead uptake and toxicity in various aquatic organisms as related to pH.

Organism	[Pb] (μmol/L)	Form[a]	pH	Medium	Observed effect of ΔpH	Reference
Selenastrum capricornutum	<15	T	8.0, 6.2	Inorganic + EDTA + Tris (defined)	Lower pH, more toxic (growth)	Monahan 1976
Trichoderma sp. *Aspergillus niger*	5×10^3 $\sim 10^4$	T T	9–5	Complex organic; solid	Lower pH, more toxic (growth)	Babich and Stotzky 1979
Lepomis gibbosus	Radiotracer	T	7.5, 6.0	Lake water	Lower pH, greater Pb uptake	Merlini and Pozzi 1977

[a]Results are expressed on the basis of the concentrations of total metal (T).

however, this trend is more surprising, since lower pH is expected to increase the free copper ion concentration in all media and very few of the cited studies were normalized for Cu^{2+} concentration.

Only limited data are available for Pb, but the opposite trend would seem to exist; at lower pH, Pb appears to be more toxic or more available for uptake. For this metal, pH-induced changes in speciation are apparently more important than any effect of H^+ competition at the level of the cell surface.

Results for Hg, again limited in number, are inconclusive; two studies (both with algae) suggest that Hg toxicity is enhanced at lower pH values, whereas in the other three investigations (alga, fungus, invertebrate) the opposite trend is reported. The available field observations indicate that fish from low-pH lakes have higher Hg levels than do those from circumneutral lakes of similar trophic status (NRCC 1981).

The studies on Al show more inconsistencies than do those involving the other metals; in fact, no conclusive trends with decreasing pH can be detected. Given that H^+ concentration may affect the solubility of Al, its speciation in solution, and its biological uptake, and that these respective interactions are likely to be complex, this result is perhaps not unexpected. In a recent study on juvenile rainbow trout, Neville (1985) proposed that the toxic effects of Al at pH 4.5 and 6.1 result from two different mechanisms at the organism level. Clearly we have at present a very limited understanding of the mechanism of Al uptake and toxicity, yet these pH–Al–organism interactions probably have greater practical importance in an environmental context than any of the others discussed here, with the possible exception of those involving Hg.

General Discussion

Having reviewed anticipated and observed variations in metal availability and toxicity to aquatic biota over the pH range 7–4, we now consider the specific questions formulated earlier.

Does the literature provide evidence that the response to metals of aquatic biota is pH dependent?

The overall response of aquatic biota to metals is very frequently pH dependent. This has been shown for 6 of the 10 metals considered: Al, Cd, Cu, Zn, and, to a lesser extent, Hg and Pb. These six metals fall into two classes: those for which a decrease in pH results in a decreased biological response (Cd, Cu, Zn), and those exhibiting the opposite behavior (Pb). Data for the remaining metals (Al, Hg) clearly reveal pH effects, but the results are too few and too inconsistent to allow generalizations.

For individual metals, are there any empirically derived pH-related patterns that agree with those expected theoretically?

As outlined previously, two contrasting types of behavior in response to acidification might be anticipated. If upon acidification the increase in the proportion of metal aquo ion is low, and if metal binding at biological surfaces is relatively weak, the overriding effect of a decrease in pH will be to attenuate metal toxicity (type I behavior), due to H^+ competition for binding sites and/or a H^+ effect on membrane potential. On the other hand, if the decrease in pH strongly affects metal speciation in solution and the metal is strongly bound at the biological interface (i.e. H^+ ineffective in competing for binding sites), then the dominant effect of acidification will be to increase metal availability (type II behavior).

Of the six metals for which a pH-dependent biological response has been documented, two (Cd, Zn) would be expected to exhibit type I behavior and in fact do so. Similarly, type II behavior would have been anticipated for two other metals (Cu, Pb) and indeed is observed for the latter, although the data base is admittedly rather meagre. That Cu conforms not to type II but to type I behavior suggests that H^+ can indeed compete effectively with Cu^{2+} for binding sites.

The predicted behavior of the remaining two metals (Al, Hg) is less obvious. Although the speciation of Al is highly pH sensitive, as a class A cation (d^0 electron configuration, nonpolarizable) it does not have a particularly high affinity for biological binding sites containing N/S-donor ligands, e.g. proteins (Williams 1981). On the other hand, Hg does have a strong affinity for such sites but the proportion of the Hg^{2+} aquo ion changes very little over the pH range 7–4; the distribution of Hg among other dissolved forms does change markedly (Table 2) but the relative biological availability of these different forms ($Hg(OH)_2$, HgOHCl, $HgCl_2$, etc.) is unknown. Experimental data for these two metals are too few to allow a meaningful comparison between results and predictions.

Do producer and consumer organisms respectively differ in their response to pH–metal combinations?

For Cd, Cu, Pb, and Zn, similar types of response are observed for both producer and consumer organisms.

If low pH does affect metal uptake or toxicity, does this happen at realistic pH and metal levels?

The effects of changing pH do indeed occur over a realistic range of H^+ concentrations (<10 μmol/L). Moreover, in many of the cited studies the metals have been used at concentrations not far removed from those found in the field, albeit in some instances in polluted situations (<10 μmol/L).

For those organisms that are inherently sensitive to pH values <6, the pH–metal interactions described here may be of only academic interest. For aquatic biota that can tolerate pH levels between 6 and 5, however, it would appear that these interactions may indeed be of practical importance; in effect, this level of acidification (pH 6–5) corresponds to that at which a number of metals are mobilized. In this context, we recommend that the chemical and biological effects of H^+–Al and H^+–Hg interactions be given particular attention.

Acknowledgments

Financial support from the Natural Sciences and Engineering Research Council (P.G.C.C. and P.M.S.), the Quebec Ministry of Intergovernmental Affairs (interprovincial university cooperation (P.G.C.C.), and the Ontario Ministry of Colleges and Universities (interprovincial university cooperation) (P.M.S.) is gratefully acknowledged. R. W. Bradley, J. B. Sprague, and B. A. Whitton provided useful insight early in the preparation of this review. Helpful comments on a draft version were furnished by J. Buffle, D. Huizenga, R. Schenck, and A. Tessier. Invaluable literature searches were performed by J. Eichmanis.

References

ANDERSON, M. A., AND A. J. RUBIN [ED.] 1981. Adsorption of inorganics at solid–liquid interfaces. Ann Arbor Science Publishers Inc., Ann Arbor, MI. 357 p.

ANDREW, R. W., K. E. BIESINGER, AND G. E. GLASS. 1977. Effects of inorganic complexing on the toxicity of copper to *Daphnia magna*. Water Res. 11: 309–315.

BABICH, H., AND G. STOTZKY. 1977. Effects of cadmium on fungi and interactions between fungi and bacteria in soil: influence of clay minerals and pH. Appl. Environ. Microbiol. 33: 1059–1066.

1979. Abiotic factors affecting the toxicity of lead to fungi. Appl. Environ. Microbiol. 38: 506–513.

1983. Influence of chemical speciation on the toxicity of heavy metals to microbiota, p. 1–46. *In* J. O. Nriagu [ed.] Aquatic toxicology. John Wiley & Sons Inc., New York, NY.

BAILEY, R. C., AND P. M. STOKES. 1984. Evaluation of filamentous algae as biomonitors of metal accumulation in softwater lakes, p. 5–26. *In* Proceedings 7th ASTM Aquatic Toxicity Symposium, ASTM Special Publ. No. 854. American Society for Testing and Materials, Philadelphia, PA.

BAKER, J. P., AND C. L. SCHOFIELD. 1982. Aluminum toxicity to fish in acidic waters. Water Air Soil Pollut. 18: 289–309.

BAKER, M. D., C. I. MAYFIELD, W. E. INNISS, AND P. T. S. WONG. 1983. Toxicity of pH, heavy metals and bisulfite to a freshwater green alga. Chemosphere 12: 34–44.

BATES, S. S., A. TESSIER, P. G. C. CAMPBELL, AND J. BUFFLE. 1982. Zinc adsorption and assimilation by *Chlamydomonas variabilis* and *Scenedesmus subspicatus* (Chlorophyceae) grown in semi-continuous culture. J. Phycol. 18: 521–529.

BEVERIDGE, T. J., AND T. JACK. 1982. Binding of an inert, cationic osmium probe to walls of *Bacillus subtilis*. J. Bacteriol. 149: 1120–1123.

BEVERIDGE, T. J., AND R. G. E. MURRAY. 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. J. Bacteriol. 127: 1502–1518.

1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. J. Bacteriol. 141: 876–887.

BORGMANN, U. 1983. Metal speciation and toxicity of free metal ions to aquatic biota, p. 47–72. *In* J. O. Nriagu [ed.] Aquatic toxicology. John Wiley & Sons Inc., New York, NY.

BRADLEY, R. W. 1984. The influence of pH, hardness and alkalinity on the acute toxicity of zinc to rainbow trout, *Salmo gairdneri*. Ph.D. dissertation, Department of Zoology, University of Guelph, Guelph, Ont. 184 p.

BRADLEY, R. W., AND J. B. SPRAGUE. 1985. The influence of pH, water hardness, and alkalinity on the acute lethality of zinc to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 42: 731–736.

BREZONIK, P. L., P. A. BRAUNER, AND W. STUMM. 1976. Trace metal analysis by anodic stripping voltammetry: effect of sorption by natural and model organic compounds. Water Res. 10: 605–612.

CAMPBELL, P. G. C., M. BISSON, J. BOISVERT, R. BOUGIE, A. TESSIER, AND J. P. VILLENEUVE. 1982. Méthodologie analytique pour déterminer la spéciation de l'aluminium dans les eaux lacustres en voie d'acidification. Université du Québec, INRS-Eau, rapport scientifique no. 145: 137 p.

CHANG, P. S. S., D. F. MALLEY, N. E. STRANGE, AND J. F. KLAVERKAMP. 1983. The effects of low pH, selenium and calcium on the bioaccumulation of ^{203}Hg by seven tissues of the crayfish, *Orconectes virilis*, p. 45–67. *In* N. K. Kaushik and K. R. Solomon [ed.] Proceedings of the Eighth Annual Aquatic Toxicity Workshop: November 2–4, 1981, Guelph, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. 1151: 255 p.

CLARK, K. L., AND B. D. LAZERTE. 1985. A laboratory study of the effects of aluminum and pH on amphibian eggs and tadpoles. Can. J. Fish. Aquat. Sci. 42: 1544–1551.

CRIST, R. H., K. OBERHOLSER, N. SHANK, AND M. NGUYEN. 1981. Nature of bonding between metallic ions and algal cell walls. Environ. Sci. Technol. 15: 1212–1217.

CRONAN, C. S., AND C. L. SCHOFIELD. 1979. Aluminum leaching response to acid precipitation: effects on high elevation watersheds in the Northeast. Science (Wash., DC) 204: 304–306.

CUSIMANO, R. F., D. F. BRAKKE, AND G. A. CHAPMAN. 1986. Effects of pH on the toxicities of cadmium, copper, and zinc to trout. Can. J. Fish. Aquat. Sci. 43. (In press)

DAVIS, R. B., S. A. NORTON, C. T. HESS, AND D. F. BRAKKE. 1983. Paleolimnological reconstruction of the effects of atmospheric deposition of acids and heavy metals on the chemistry and biology of lakes in New England and Norway. Hydrobiologia 103: 113–123.

DICKSON, W. 1978. Some effects of the acidification of Swedish lakes. Verh. Int. Ver. Limnol. 20: 851–856.

1980. Properties of acidified waters, p. 75–83. *In* D. Drablos and A. Tollan [ed.] Ecological impact of acidic precipitation. SNSF Project, Oslo, Norway.

DOYLE, R. J., T. H. MATTHEWS, AND U. N. STREIPS. 1980. Chemical basis for selectivity of metals ions by the *Bacillus subtilis* cell wall. J. Bacteriol. 143: 471–480.

ENGEL, D. W., W. G. SUNDA, AND B. A. FOWLER. 1981. Factors affecting trace metal uptake and toxicity to estuarine organisms. I. Environmental parameters, p. 127–144. *In* F. J. Vernberg, A. Calabrese, F. P. Thurberg and W. B. Vernberg [ed.] Biological monitoring of marine pollutants. Academic Press, New York, NY.

ERNST, R., H. E. ALLEN, AND K. H. MANCY. 1975. Characterization of trace metal species and measurement of trace metal stability constants by electrochemical techniques. Water Res. 9: 969–979.

FERGUSON, J., AND B. BUBELA. 1974. The concentration of Cu(II), Pb(II) and Zn(II) from aqueous solutions by particulate algal matter. Chem. Geol. 13: 163–186.

FLORENCE, T. M. 1982. The speciation of trace elements in waters. Talanta 29: 345–364.

FLORENCE, T. M., AND G. E. BATLEY. 1980. Chemical speciation in natural waters. Crit. Rev. Anal. Chem. 9: 219–296.

GARDINER, J. 1974. The chemistry of cadmium in natural water — I. A study of cadmium complex formation using the cadmium specific-ion electrode. Water Res. 8: 23–30.

GIPPS, J. F., AND B. A. W. COLLER. 1980. Effects of physical and culture conditions on uptake of cadmium by *Chlorella pyrenoidosa*. Aust. J. Mar. Freshwater Res. 31: 747–755.

GOULD, M. S., AND E. J. GENETELLI. 1978a. Heavy metal complexation behavior in anaerobically digested sludges. Water Res. 12: 505–512.

1978b. The effect of methylation and hydrogen ion concentration on heavy metal binding by anaerobically digested sludges. Water Res. 12: 889–892.

1984. Effects of competition on heavy metal binding by anaerobically digested sludges. Water Res. 18: 123–126.

GUY, R. D., AND C. L. CHAKRABARTI. 1976a. Analytical techniques for speciation of heavy metal ions in the aquatic environment, p. 26–29. *In* Chemistry in Canada, November. Chemical Institute of Canada, Ottawa, Ont.

1976b. Studies of metal–organic interactions in model systems pertaining to natural waters. Can. J. Chem. 54: 2600–2611.

GUY, R. D., C. L. CHAKRABARTI, AND L. L. SCHRAMM. 1975. The application of a simple chemical model of natural waters to metal fixation in particulate matter. Can. J. Chem. 53: 661–669.

HARDING, J. P. C., AND B. A. WHITTON. 1977. Environmental factors reducing the toxicity of zinc to *Stigeoclonium tenue*. Br. Phycol. J. 12: 17–21.

HARGREAVES, J. W., AND B. A. WHITTON. 1976. Effect of pH on tolerance of *Hormidium rivulare* to zinc and copper. Oecologia (Berl.) 26: 235–243.

HARRISON, G. I., P. G. C. CAMPBELL, AND A. TESSIER. 1986. Effects of H^+-ion activity on zinc uptake by *Chlamydomonas variabilis* grown in batch culture. Can. J. Fish. Aquat. Sci. 43. (In press)

HARRISON, G. I., AND F. M. M. MOREL. 1983. Antagonism between cadmium and iron in the marine diatom *Thalassiosira weissflogii*. J. Phycol. 19: 495–507.

HART, B. A., AND B. D. SCAIFE. 1977. Toxicity and bioaccumulation of cadmium in *Chlorella pyrenoidosa*. Environ. Res. 14: 401–413.

HASSETT, J. M., J. C. JENNETT, AND J. E. SMITH. 1981. Microplate technique for determining accumulation of metals by algae. Appl. Environ. Microbiol. 41: 1097–1106.

HELLIWELL, S., G. E. BATLEY, T. M. FLORENCE, AND B. G. LUMSDEN. 1983. Speciation and toxicity of aluminum in a model fresh water. Environ. Technol. Lett. 4: 141–144.

HOWARTH, R. S., AND J. B. SPRAGUE. 1978. Copper lethality to rainbow trout in waters of various hardness and pH. Water Res. 12: 455–462.

JACKSON, T. A., G. KIPPHUT, R. H. HESSLEIN, AND D. W. SCHINDLER. 1980. Experimental study of trace metal chemistry in soft-water lakes at different pH levels. Can. J. Fish. Aquat. Sci. 37: 387–402.

JENNE, E. A. [ED.] 1979. Chemical modeling in aqueous systems. A.C.S. Symp. Ser. No. 93. American Chemical Society, Washington, DC. 893 p.

JOHNSON, N. M., C. T. DRISCOLL, J. S. EATON, G. E. LIKENS, AND W. H. MCDOWELL. 1981. Acid rain, dissolved aluminum and chemical weathering at the Hubbard Brook Experimental Forest, New Hampshire. Geochim. Cosmochim. Acta 45: 1421–1437.

KAISER, K. L. E. 1980. Correlation and prediction of metal toxicity to aquatic biota. Can. J. Fish. Aquat. Sci. 37: 211–218.

KERNDORFF, H., AND M. SCHNITZER. 1980. Sorption of metals on humic acid. Geochim. Cosmochim. Acta 44: 1701–1708.

KINSEY, N. 1975. Effets du pH sur le dosage des métaux traces par la méthode de la polarographie inverse. Thèse de maîtrise es sciences (EAU), Université du Québec, INRS-Eau, Sainte-Foy, Qué. 133 p.

LACHANCE, M., B. BOBÉE, AND Y. GRIMARD. 1985. Sensitivity of southern Quebec lakes to acidic precipitation. Water Air Soil Pollut. 25: 115–132.

LES, A., AND R. W. WALKER. 1984. Toxicity and binding of copper, zinc, and cadmium by the blue-green alga, *Chroococcus paris*. Water Air Soil Pollut. 23: 129–139.

MAGNUSON, V. R., D. K. HARRISS, M. S. SUN, D. K. TAYLOR, AND G. E. GLASS. 1979. Relationships of activities of metal–ligand species to aquatic toxicity, p. 635–656. *In* E. A. Jenne [ed.] Chemical modeling in aqueous systems. A.C.S. Symp. Ser. No. 93. American Chemical Society, Washington, DC.

MARSHALL, J. S., J. I. PARKER, D. L. MELLINGER, AND S. G. LAWRENCE. 1981. An in situ study of cadmium and mercury stress in the plankton community of Lake 382, Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 38: 1209–1214.

MATTHEWS, T. H., R. J. DOYLE, AND U. N. STREIPS. 1979. Contribution of peptidoglycan to the binding of metal ions by the cell wall of *Bacillus subtilis*. Curr. Microbiol. 3: 51–53.

MCBRIDE, M. B., AND D. R. BOULDIN. 1984. Long-term reactions of copper(II) in a contaminated calcareous soil. Soil Sci. Soc. Am. J. 48: 56–59.

MERLINI, M., AND G. POZZI. 1977. Lead and freshwater fishes — Part 1: Lead accumulation and water pH. Environ. Pollut. 12: 167–172.

MIERLE, G. 1982. Studies on the uptake and toxicity of copper and other transition metal ions with a green alga, *Scenedesmus acuminatus*. Ph.D. dissertation, Department of Botany, University of Toronto, Toronto, Ont. 248 p.

MIERLE, G., AND P. M. STOKES. 1976. Heavy metal tolerance and metal accumulation by planktonic algae, p. 113–122. *In* D. P. Hemphill [ed.] Trace substances in environmental health. Vol. X. University of Missouri, Columbia, MO.

MILLER, T. G., AND W. C. MACKAY. 1980. The effects of hardness, alkalinity and pH of test water on the toxicity of copper to rainbow trout (*Salmo gairdneri*) Water Res. 14: 129–133.

MONAHAN, T. J. 1976. Lead inhibition of chlorophycean microalgae. J. Phycol. 12: 358–362.

MOREL, F. M. M., AND N. M. L. MOREL-LAURENS. 1983. Trace metals and plankton in the oceans: facts and speculations, p. 841–869. *In* C. S. Wong, E. Boyle, K. W. Bruland, J. D. Burton, and E. D. Goldberg [ed.] Trace metals in sea water. Plenum Press, New York, NY.

MÜLLER, K. W., AND H.-D. PAYER. 1979. The influence of pH, zinc, and light on the cadmium-repressed growth of the green alga *Coelastrum proboscideum*, p. 296–299. *In* Proceedings, Int. Conf. Management Control Heavy Metals in the Environment, London. CEP Consultants, Edinburgh, U.K.

NAKAJIMA, A., T. HORIKOSHI, AND T. SAKAGUCHI. 1979. Uptake of manganese ion by *Chlorella regularis*. Agric. Biol. Chem. 43: 1461–1466.

NELSON, P. O., A. K. CHUNG, AND M. C. HUDSON. 1981. Factors affecting the fate of heavy metals in the activated sludge process. J. Water Pollut. Control Fed. 53: 1323–1333.

NEVILLE, C. 1985. Studies on the mechanism of the toxicity of acid and aluminum to juvenile rainbow trout in a low ion environment. Int. Symp. Acidic Precipitation, Muskoka, Ontario, 15–20 Sept. 1985, Abstracts, p. 143.

NORDSTROM, D. K., L. N. PLUMMER, J. M. L. WIGLEY, T. J. WOLERY, J. W. BALL, E. A. JENNE, R. L. BASSETT, D. A. CRERAR, T. M. FLORENCE, B. FRITZ, M. HOFFMAN, G. R. HOLDREN, G. M. LAFON, S. V. MATTIGOD, R. E. MCDUFF, F. MOREL, M. M. REDDY, G. SPOSITO, AND J. THRAILKILL. 1979. A comparison of computerized chemical models for equilibrium calculations in aqueous systems, p. 857–892. *In* E. A. Jenne [ed.] Chemical modeling in aqueous systems. A.C.S. Symp. Ser. No. 93. American Chemical Society, Washington, DC.

NRCC. 1981. Acidification in the Canadian Environment. National Research Council of Canada, Assoc. Committee Sci. Criteria Environ. Quality, Report No. NRCC 18475: 369 p.

O'SHEA, T. A., AND K. H. MANCY. 1978. The effect of pH and hardness metal ions on the competitive interaction between trace metal ions and inorganic

and organic complexing agents found in natural waters. Water Res. 12: 703–711.

PAGENKOPF, G. K. 1983. Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH, and water hardness. Environ. Sci. Toxicol. 17: 347–352.

PERDUE, E. M., AND C. R. LYTLE. 1983. A critical examination of metal–ligand complexation models: application to defined multiligand mixtures, p. 295–313. *In* R. F. Christman and E. T. Gjessing [ed.] Aquatic and terrestrial humic materials. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

PETERSON, H. G., F. P. HEALEY, AND R. WAGEMANN. 1984. Metal toxicity to algae: a highly pH dependent phenomenon. Can. J. Fish. Aquat. Sci. 41: 974–979.

PETERSEN, R. 1982. Influence of copper and zinc on the growth of a freshwater alga, *Scenedesmus quadricauda*: the significance of chemical speciation. Environ. Sci. Technol. 16: 443–447.

RAI, L. C., J. P. GAUR, AND H. K. KUMAR. 1981. Protective effects of certain environmental factors on the toxicity of zinc, mercury and methylmercury to *Chlorella vulgaris*. Environ. Res. 25: 250–259.

RAVEN, J. A. 1980. Nutrient transport in microalgae, p. 47–226. *In* A. H. Rose and J. G. Morris [ed.] Advances in microbial physiology. Vol. No. 21, Academic Press, New York, NY.

RICHMAN, L. A., AND P. M. STOKES. 1985. The effect of experimental acidification on the bioaccumulation of methylmercury in an aquatic food chain. Int. Symp. Acidic Precipitation, Muskoka, Ontario, 15–20 Sept. 1985, Abstracts, p. 110–111.

SAKAGUCHI, T., T. TSUJI, A. NAKAJIMA, AND T. HORIKOSHI. 1979. Accumulation of cadmium by green microalgae. Eur. J. Appl. Microbiol. Biotechnol. 8: 207–215.

SCHINDLER, D. W., R. H. HESSLEIN, R. WAGEMANN, AND W. S. BROECKER. 1980. Effects of acidification on mobilization of heavy metals and radionuclides from sediments of a lake. Can. J. Fish. Aquat. Sci. 37: 373–377.

SCHONBERGER, E. A., AND W. F. PICKERING. 1980. The influence of pH and complex formation on the ASV peaks of Pb, Cu and Cd. Talanta 27: 11–18.

SCOTT, I. T. 1926. Some protein analogies of the mycelium of *Fusarium lycopersici*. Missouri Agr. Exp. Stn. Res. Bull. 92: 3–44.

1929. Hydrogen-ion equilibrium of mycelial mats of *Fusarium lycopersici* in salt solutions and its relation to growth and toxicity. Am. J. Bot. 16: 641–643.

SHEHATA, F. H. A., AND B. A. WHITTON. 1982. Zinc tolerance in strains of the blue-green alga *Anacystis nidulans*. Br. Phycol. J. 17: 5–12.

SHUMAN, M. S., AND L. C. MICHAEL. 1978. Reversibility of copper in dilute aqueous carbonate and its significance to anodic stripping voltammetry of copper in natural waters. Anal. Chem. 50: 2104–2109.

SINGH, N. 1977. Effect of pH on the tolerance of *Penicillium nigricans* to copper and other heavy metals. Mycologia 69: 750–755.

SPOSITO, G. 1981. Trace metals in contaminated wastes. Environ. Sci. Technol. 15: 396–403.

SPRAGUE, J. B. 1964. Lethal concentrations of copper and zinc for young Atlantic salmon. J. Fish. Res. Board Can. 21: 17–26.

STARKEY, R. C. 1973. Effect of pH on toxicity of copper to *Scytalidium* sp., a copper-tolerant fungus, and some other fungi. J. Gen. Microbiol. 78: 217–225.

STARÝ, J., B. HAVLÍK, K. KRATZER, J. PRÁŠILOVÁ, AND J. HANUŠOVÁ. 1983a. Cumulation of zinc, cadmium and mercury on the alga *Scenedesmus obliquus*. Acta Hydrochim. Hydrobiol. 11: 401–409.

STARÝ, J., AND K. KRATZER. 1982. The cumulation of toxic metals on alga. J. Environ. Anal. Chem. 12: 65–71.

STARÝ, J., K. KRATZER, AND J. PRÁŠILOVÁ. 1983b. Systematic study of the cumulation of elements on algae. Toxicol. Environ. Chem. 7: 47–61.

STAUB, C., J. BUFFLE, AND J. HAERDI. 1984. Measurement of complexation properties of metal ions in natural conditions by ultrafiltration: influence of various factors on the retention of metals and ligands by neutral and negatively charged membranes. Anal. Chem. 56: 2843–2849.

STEEMANN NIELSEN, E., AND L. KAMP-NIELSEN. 1970. The influence of deleterious concentrations of copper on the growth of *Chlorella pyrenoidosa*. Physiol. Plant. 23: 828–840.

STEEMANN NIELSEN, E., L. KAMP-NIELSEN, AND S. WIUM-ANDERSEN. 1969. The effect of deleterious concentrations of copper on the photosynthesis of *Chlorella pyrenoidosa*. Physiol. Plant. 22: 1121–1133.

STUMM, W., AND J. J. MORGAN. 1981. Aquatic chemistry. An introduction emphasizing chemical equilibria in natural waters. John Wiley & Sons Inc., New York, NY. 780 p.

SUNDA, W. G., AND S. A. HUNTSMAN. 1983. Effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. Limnol. Oceanogr. 28: 924–934.

WAIWOOD, K. G., AND F. W. H. BEAMISH. 1978. Effects of copper, pH and hardness on the critical swimming performance of rainbow trout (*Salmo gairdneri*). Water Res. 12: 611–619.

WANG, H.-K., AND J. M. WOOD. 1984. Bioaccumulation of nickel by algae. Environ. Sci. Technol. 18: 106–109.

WESTALL, J. C., J. L. ZACHARY, AND F. M. M. MOREL. 1976. MINEQL, a computer program for the calculation of the chemical equilibrium composition of aqueous systems. MIT Dep. Civ. Eng. Tech. Rep. 18: 91 p.

WILLIAMS, R. J. P. 1981. Physico-chemical aspects of inorganic element transfer through membranes. Phil. Trans. R. Soc. Lond. B 294: 57–74.

WOOD, J. M. 1974. Biological cycles for toxic elements in the environment. Science (Wash., DC) 183: 1049–1052.

WRIGHT, R. F., AND E. SNEKVIC. 1978. Acid precipitation: chemistry and fish populations in 700 lakes in southernmost Norway. Verh. Int. Ver. Limnol. 20: 765–775.

YAN, N. D., AND P. M. STOKES. 1978. Phytoplankton in an acidic lake, and its responses to experimental alterations of pH. Environ. Conserv. 5: 93–100.

NOTES

The Right to Fish as an External Benefit of Open Access

Ralph E. Townsend

Department of Economics, University of Maine at Orono, Orono, ME 04469, USA

Townsend, R. E. 1985. The right to fish as an external benefit of open access. Can. J. Fish. Aquat. Sci. 42: 2050–2053.

Work is more than a means to earn income. Work activity is intimately related to the values of a society. This is especially true for occupational communities. Fishing activity may have very profound effects upon the social system. Open access to the resource is one dimension that reinforces this connection. A worker satisfaction bonus is much too narrow a concept to capture the depth of the relationship of social benefits to the open-access institution. To understand the role that the birthright performs, the entire ecological system — biological, economic, political, and social — in which fishermen work and live must be understood.

Le travail n'est pas seulement une source de revenus; il est intimement lié aux valeurs sociales. Cela est particulièrement vrai dans les collectivités à vocation professionnelle. La pêche peut avoir des effets très marqués sur le système social. Le libre accès à la ressource est un des facteurs qui renforcent cette relation. Le concept de la gratification par la satisfaction du travail est un concept trop étroit pour définir l'importance de la relation entre les avantages sociaux et le libre accès. Il faut, pour connaître le rôle joué par le patrimoine, tout d'abord connaître le système écologique global – biologique, économique, politique et social – dans lequel vivent et travaillent les pêcheurs.

Received May 3, 1984
Accepted July 31, 1985
(J7772)

Reçu le 3 mai 1984
Accepté le 31 juillet 1985

The conventional wisdom of fisheries management now leans heavily toward limited entry as the management of choice. The restricted right to fish under limited entry becomes a substitute for the absent property rights to the resource. The economic philosophy of creating property rights as a solution to the common property problem may be traced to Scott's (1955) analysis of the "sole owner." The concept of limited entry reached its preeminent position in fisheries economics not only because it addressed the fundamental dilemma of unlimited access, but also because the economic rents generated remained with fishermen. One of the earliest discussions of the practical and theoretical issues in the implementation of limited entry was Sinclair's (1961) prescription for the British Columbia salmon fisheries.

Biologists, by and large, came to support limited entry as a desirable component of any management program. This biological support reflected two practical, political advantages of limited entry. Limited entry is a relatively permanent limit on fishing effort, so the perennial battles over administrative limits on effort could be largely avoided. Moreover, limited entry automatically created a pool of political supporters for management: the limited entry license holders.

Limited entry has been widely implemented in both Canada and Australia (see Smith 1978 and Sturgess and Meany 1982, respectively). In the United States and in Europe, limited entry has been much less evident. With the enactment of the U.S. Fisheries Conservation and Management Act of 1976, however, a virtually nationwide debate on limited entry did erupt in the United States. Political opposition from American fishermen was widespread, and only a few limited entry plans were actually adopted.

This political opposition to limited entry could not be easily dismissed. If the theoretical beneficiaries strongly oppose limited entry, then some flaw must exist in the underlying theory. Several criticisms of the economic theory arose to explain the anomaly. For example, Bromley and Bishop (1977) focussed upon the weak welfare foundations of the economic theory. Wilson (1982) criticized the theory as fundamentally too simple to explain behavior in the multispecies fisheries. Johnson and Libecap (1982) suggested that limited entry may alter the distribution of quasi-rents among fishermen.

The opposition to limited entry also might be explained by noneconomic benefits that fishermen receive from fishing. If the value of nonpecuniary sacrifices exceeded the pecuniary benefits of limited entry, then fishermen would rationally oppose limited entry. Anderson (1980) introduced the "worker satisfaction bonus" to analyze the nonpecuniary benefits that fishermen receive. Anderson showed that, even with a worker satisfaction

bonus, the level of effort in an open-access fishery is unambiguously higher than the economically optimal level. Of course, the optimal level does depend upon the extent of the worker satisfaction in fisheries and in alternative employment. That analysis would seem to argue that the fundamental case for limited entry is unaltered by nonpecuniary benefits. The present paper argues that that conclusion is based upon a very narrow concept of nonpecuniary benefits.

A Broader Concept of Nonpecuniary Benefits

Anderson's worker satisfaction bonus is a special case of nonpecuniary benefits. There is an important difference between the satisfaction a fisherman enjoys while pursuing his livelihood and the value that fishermen and others place upon the unrestricted right to fish. The worker satisfaction bonus is linked directly to the activity of fishing. The right to fish may be valued even by those who do not fish. Moreover, management that ends open access may reduce the welfare of those who continue to fish when descendants, relatives, and friends are denied access. Anderson's analysis addresses only the activities linked to fishing directly, and not to the birthright or related social benefits of open access. When a broader concept of nonpecuniary benefits from open access is applied, effort reduction by limited entry is not necessarily optimal.

The idea that society in general values an economic institution might be dismissed as a variation on the trivial argument that any institutional change involves some transition costs. That is not my argument. Rather, for communities whose economic activities depend heavily upon fisheries, the right to fish is an important component of the social structure. Ending open access may dramatically alter the entire social environment. Somewhat more narrowly, the right to fish provides nonfishermen with potential opportunities that are valued. In Weisbrod's (1964) terms, there is an "option value" associated with the right to fish.

Social scientists have tended to separate the study of economic activity from other social activity. As a result of this compartmentalization, the interrelationships between economic and noneconomic life are often overlooked. The theme that economic life is inherently interconnected with the rest of social activity goes back at least to Marx. More directly, Hughes (1958) argued that the conditions of work and employment affect both the individual personality and also social relationships. Hughes' contribution laid the foundations for the study of the sociology of work and occupations.

Among the studies of the sociology of work, Salaman's (1974) study of "occupational communities" is especially relevant to the relationship between fishing as work and fishing as a way of life. In these occupational communities, "Members of occupational communities are affected by their work in such a way that their non-work lives are permeated by their work relationships, interests and values" (Salaman 1974, p. 19). Salaman identified three features of an occupational community: (1) the self-image of the worker is centered on the occupational role; (2) members share a reference group composed of members of the occupational community; (3) members associate with other members of the occupational community in preference to outsiders.

Workers do not simply decide to form occupational communities. Rather, something about the nature of the work and the work environment ties workers together. Salaman suggested three specific determinants: (i) worker involvement in the job, (ii) a discrepancy between the social and occupational assessment of the status of workers, and (iii) the inclusiveness of the job role.

Two of these concepts require some elaboration. Salaman thought worker involvement was determined by the danger in the work environment, the degree of skill required, the level of autonomy of the worker, any extreme or unpleasant work conditions, and the social importance of the work. A job is inclusive if demands of the work role distinguish the worker's lifestyle from the social norm. Odd working hours and extended time away from home are examples of inclusive job demands.

Given the special conditions of job involvement and job inclusiveness for fishermen, it is not surprising that most fishermen do belong to occupational communities. Fishermen thus are different from most industrial workers, who explicitly separate their work from their nonwork lives. Work as a fisherman is not "just another job"; work for a fisherman is a fundamental part of his way of life.

The occupational community of fishermen is often closely connected to another identifiable community. This close connection may be created by the geographical isolation of small fishing villages, or by the unique cultures of ethnic groups, such as the Portuguese and Italians in San Diego's tuna seiners (Orbach 1977) and in the trawler fleet of Gloucester (Bartlett 1977). In such communities, the social attitudes of fishermen shape broader social attitudes. The entire community has a stake in the fishing industry, even when some members are not employed in fishing or fishing-related jobs.

The unique relationship between fisheries and the social environment has been noted by a number of cultural anthropologists. Two studies, Norr and Norr (1974) and Poggie and Gersuny (1974), provide some particularly relevant analysis.

Norr and Norr (1974) compared the social position of laborers in fishing and in agriculture in 11 complex peasant agricultural societies. They concluded that the unique technical and environmental demands of fishing dramatically alter the social relationships of fishermen. The three factors they identified as differentiating fishing from agriculture are the physical separation of the worksite from the community, the high level of interdependence of fishing crews, and the greater risk. The result of these unique demands is a more equal employer–employee relationship, greater control by employees over working conditions, and a higher economic reward. Although Norr and Norr were analyzing peasant societies, their results would seem to apply equally to modern fisheries.

Poggie and Gersuny (1974) compared the social attitudes of fishermen in Galilee (Point Judith), Rhode Island, and of mill workers in New Bedford, Rhode Island. Some rather dramatic differences appeared. Fishermen have a much more positive self-image. Fishermen are much more likely to approve of a son's decision to adopt his father's career than are millworkers. Patrilateral kinship ties are much more important among fishermen. Poggie and Gersuny attributed the importance of patrilateral kinship to the relative dominance of instrumental values (which are traditionally male roles) over expressive values (which are traditionally female roles) in fishing communities. Poggie and Gersuny concluded that "self-realization is associated more with work than with family life *per se* among fishermen than among mill workers." The stark contrast between the values of the two groups illustrates the interconnection between fishing and social values.

My interest in the relationship between fishing and social structures was motivated by personal observation of the fishing

communities of eastern Maine. The social attitudes and relationships in these communities illustrate that fishing under conditions of open access is important to the entire community.

Employment in these generally isolated Maine communities relies heavily upon three open-access fisheries: soft-shell clams, lobsters, and herring. A number of other open-access fisheries also contribute to employment at least seasonally: scallops, shrimp, mussels, and groundfish.[1] In the clam fishery, only a very small investment (perhaps U.S.$50) is required, so literally any ablebodied person can dig clams. The other fisheries require more substantial investment for boat and gear, but this investment is within the financial means of a large part of the population. In the local jargon, one "can make a living" in these fisheries, although the work is hard and the economic rewards rather modest.

The availability of fishing employment has considerable impact upon the social structure of these Maine towns. Young men who want to remain in their hometowns can earn a living. The employment does not, however, attract significant inmigration. The resultant labor market is quite localized, and this local economic focus contributes to a social structure that is relatively stable over time. Not surprisingly, the local attitudes do not approve of welfare for ablebodied men as long as the fisheries are available. Employer–employee relations maintain a tenor of mutual respect, at least in part because self-employment is immediately available for many employees. Because much of the community is self-employed, political equality is reinforced by relative economic equality. Unlike most of contemporary American society, these communities retain the rural, agrarian character that spawned American political ideology.

These examples really expand Salaman's concept of an occupational community. Not only do fishermen share a reference set of values, but these values affect the entire social system. This broad influence occurs not only because a substantial part of the workforce fishes, but also because a birthright guarantees fishing as an option for everyone. When access is limited, the social system is altered not only because fewer men may fish, but also because an important common bond within the community will be ended. The values of the occupational community of fishermen become the values of the privileged elite with licenses, rather than common community values.

Any program to limit access to the resource can affect the entire community. Such losses are unrelated to economic variables, such as revenues, costs, or economic surpluses. This loss may be greater than any potential improvement in economic efficiency. Unlike the simple worker satisfaction bonus, a theoretical presumption in favor of limited access cannot be demonstrated. The relative merit of management based upon limited access becomes strictly an empirical question: Do social benefits exceed social costs?

Towards a Research Agenda

The concept of a right to fish is obviously troubling to proponents of limited entry. To acknowledge the most extreme forms of a birthright is to reject limited entry by tautology. But to ignore the role of such a birthright overlooks the fundamental social concerns of those who are to benefit from limited entry. Neither extreme is justified. The birthright and its relation to the social order are additional features of the very complicated system that must be addressed by rational management. This system includes the biological environment, the economic environment, the political environment, and also the social environment.

Biologists and economists, who are the traditional fishery scientists, are generally ill-suited to analyzing the roots and functions of the birthright to fish. Not only is the subject unfamiliar, but also the issues do not lend themselves to the strict scientific process of gathering empirical data and testing rigorous statistical models. The task requires the research skills and professional judgement of political scientists, cultural anthropologists, and sociologists. To deal with this social issue, economists and biologists must yield part of their virtual monopoly in fishery research to other social scientists.

To incorporate the right of access and related social institutions into fishery management, a three-part task must be completed. First, the exact nature of the right must be understood. Second, the relation between the right and socioeconomic institutions must be explained. Finally, the practical issue of how management should best accommodate this right must be elaborated.

In defining the right of access, a clear distinction must be made between the existing legal rights and underlying community definition of rights. The legal structure invariably gives anyone who meets minimal residency requirements the right of access. The community, however, may substantially alter the de jure rights with de facto limitations. Such de facto definitions may be evident in cooperative or noncooperative behavior towards new fishermen by existing fishermen. Established residents usually have a preeminent claim. The claim may be strongest for members of families with fishing backgrounds. The right may be forfeited by the violation of implicit obligations or by the decision to pursue nonfishing employment for an extended period. Within any community, the definition of rights may vary from fishery to fishery. When conflicting claims to the same rights exist, an unwritten constitution may arbitrate these claims. Acheson (1972) and Acheson and Lazarowitz (1979) discussed the types of social norms that define the birthright to fish for lobsters in various Maine communities. These studies typify the research required to understand the existing social institutions.

The right to fish is not an isolated phenomenon, unrelated to the rest of society. Rather, the right of access is part of an integrated socioeconomic framework. To understand the relation between the birthright and broader social institutions, questions such as the following must be asked: Is the birthright unique to isolated, socially static communities, or does this right also exist in mobile, metropolitan communities? Is the nature of the right of access related to the economic dependence of the community upon fisheries? Is the right associated with particular social attitudes toward family, community, and nonresidents? Do the characteristics of the resource itself alter the type of right bestowed?

Finally, research must identify management alternatives that will minimize the disruption of existing social institutions. Such management alternatives may include limitations on who may hold a license, restrictions on license transfer, or apprenticeship requirements. Although such restrictions are often criticized as economically inefficient, they may well be socially desirable. Programs to reduce economic dependence upon fisheries, such as local economic development and manpower training programs, may also reduce the social cost of ending open access.

[1]Although all these fisheries are open to local residents, there are legal barriers to nonresidents in some clam fisheries and extralegal barriers in lobster fisheries (Acheson 1972). The location of fixed herring gear is also subject to local political control.

A birthright institution is not necessarily a roadblock to management by limited entry. When management is based upon a definition of rights that is closer to the social perspective than existing laws, management may find substantial support. Moreover, the birthright often carries obligations to husband and conserve the resource. Anthropologists have documented a wide array of nonmarket institutions that determine both the rights and obligations for the use of common property resources. Examples include Oceania (Johannes 1978), Alaskan salmon fisheries (Rogers 1972), and the lobstermen of Maine (Acheson 1972).

Thus, the incorporation of a worker satisfaction bonus into economic models does not exhaust the nonmonetary benefits derived from an open-access fishery. The very institution of open access may be valuable even to those who do not fish, and when the entire community values the right, effort reduction by limited entry may not be the optimal social program.

Acknowledgements

This work was supported by NOAA, National Sea Grant Program, Department of Commerce, under grant No. NA81AA-D-0035 through the Maine – New Hampshire Sea Grant Program. The idea presented here has benefitted from extensive discussion with James Acheson and James Wilson.

References

ACHESON, J. M. 1972. The territories of the lobstermen. Nat. Hist. 81: 60–69.

ACHESON, J. M., AND T. LAZAROWITZ. 1979. Using the family jewels: the family estate in coastal Maine. *In* J. M. Achesom [ed.] Essays on social and cultural aspects of New England fisheries: implications for management. (Final report for NSF grant AER 77-06018). University of Maine, Orono, ME. 842 p.

ANDERSON, L. G. 1980. Necessary components of economic surplus in fisheries economics. Can. J. Fish. Aquat. Sci. 37: 858–870.

BARTLETT, K. 1977. The finest kind. Norton, New York, NY.

BROMLEY, D. W., AND R. C. BISHOP. 1977. From economic theory to fisheries policy: conceptual problems and management prescriptions. *In* L. G. Anderson [ed.] Economic impacts of extended fisheries jurisdictions. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

HUGHES, E. C. 1958. Men and their work. Free Press, Glencoe, IL.

JOHANNES, R. E. 1978. Traditional marine conservation methods in Oceania and their demise. Annu. Rev. Ecol. Syst. 9: 349–364.

JOHNSON, R. N., AND G. D. LIBECAP. 1982. Contracting problems and regulation: the case of a fishery. Am. Econ. Rev. 72: 1005–1022.

NORR, K. L., AND J. L. NORR. 1974. Environmental and technical factors influencing power in work organizations. Soc. Work Occup. 1: 219–251.

ORBACH, M. K. 1977. Hunters, seamen, and entrepreneurs. University of California Press, Berkeley, CA.

POGGIE, J., AND C. GERSUNY. 1974. Fishermen of Galilee. University of Rhode Island Marine Bulletin 17, Kingston, RI.

ROGERS, G. W. 1972. Fisheries management: the Cook Inlet and Bristol Bay cases. In A. R. Tussing, T. A. Morehouse, and J. D. Babb [ed.] Alaska fisheries policy. Inst. Soc. Econ. Gov. Res., Fairbanks, AK.

SALAMAN, G. 1974. Community and occupation. Cambridge University Press, London.

SCOTT, A. D. 1955. The fishery: the objectives of sole ownership. J. Pol. Econ. 63: 116–124.

SINCLAIR, S. 1961. License limitation — a method of economic fisheries management. Department of Fisheries and Oceans, Ottawa, Ont.

SMITH, L. J. 1978. Case studies on economic effects of limiting entry to the fisheries. *In* R. B. Rettig and J. C. Ginter [ed.] Limited entry as a fishery management tool. University of Washington Press, Seattle, WA.

STURGESS, N. H., AND T. F. MEANY. 1982. Policy and practice in fisheries management. Australian Government Publ., Canberra, Australia.

WEISBROD, B. 1964. Collective consumption services of individual consumption goods. Q. J. Econ. 78: 471–477.

WILSON, J. A. 1982. The economical management of multispecies fisheries. Land Econ. 58: 417–434.

Removal of Follicle Wall Components from Ovarian Oocytes of the Brook Trout, *Salvelinus fontinalis*

Charles A. Lessman

Department of Biology, St. Francis Xavier University, Antigonish, N.S. B2G 1C0

Soosamma Kavumpurath

Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1

and Hamid R. Habibi

Department of Biology, St. Francis Xavier University, Antigonish, N.S. B2G 1C0

Lessman, C. A., S. Kavumpurath, and H. R. Habibi. 1985. Removal of follicle wall components from ovarian oocytes of the brook trout, *Salvelinus fontinalis*. Can. J. Fish. Aquat. Sci. 42: 2053–2058.

Fully grown brook trout (*Salvelinus fontinalis*) ovarian follicles, preincubated in Ca^{2+}-free media, were dissected from other ovarian tissue by use of watchmaker forceps. Manual defolliculation and further Ca^{2+}-free treatments were used to obtain oocytes freed of one or more follicular investments. Combinations of these treatments ultimately resulted in oocytes denuded of somatic cells as demonstrated by scanning electron microscopy. Mechanically denuded brook trout oocytes maintained the ability to undergo progestogen-induced meiotic maturation in vitro. This technique, which avoids use of enzymes that may alter cell surfaces, provides a useful system for the study of interactions between follicle wall components and the oocyte during hormonally induced meiotic maturation in vitro.

Des follicules ovariens d'omble de fontaine (*Salvelinus fontinalis*) matures, pré-incubés en milieu exempt de Ca^{2+}, ont été isolés des autres tissus ovariens à l'aide de pincettes d'horloger. L'isolement manuel des follicules suivi d'autres traitements en milieu exempt de Ca^{2+} a permis d'obtenir des oocytes débarrassés d'une ou de plusieurs couches folliculaires. L'utilisation combinée de ces traitements a permis d'obtenir des oocytes exempts de cellules somatiques, comme cela a été démontré par microscopie électronique à balayage. Les oocytes d'omble de fontaine mécaniquement dénudés conservaient leur capacité de maturation méiotique progestinogène in vitro. Cette technique, qui évite d'utiliser des enzymes pouvant altérer les surfaces cellulaires, pourra s'avérer utile à l'étude des interactions entre les composantes des parois folliculaires et l'oocyte au cours de la maturation méiotique hormonale in vitro.

Received February 7, 1985
Accepted August 13, 1985
(J8111)

Reçu le 7 février 1985
Accepté le 13 août 1985

The teleost ovarian follicle is a complex consisting of somatic cells and a single germ cell or oocyte (Guraya 1978). The somatic cells in the brook trout (*Salvelinus fontinalis*) ovarian follicle are arranged in three main layers. The outermost is the ovarian epithelium (oe), the next layer is the theca folliculi (tf), and the layer adjacent to the oocyte is the follicle cell epithelium (fe) (Flugel 1967). Basement membranes (BM) separate the three layers. Recent evidence indicates different roles for these three layers in salmonids, especially with respect to steroid biosynthesis and metabolism (Kagawa et al. 1982). In leopard frogs (*Rana pipiens*), Lessman and Schuetz (1981, 1982) found that exogenously applied hormones differentially effect oocyte meiosis reinitiation depending upon the presence or absence of follicle wall components in ovarian follicles. In their review, Masui and Clarke (1979) pointed out the paucity of studies involving follicle-free oocytes in fish. This may reflect the lack of published techniques for follicle wall removal in this group.

Questions relating to follicle wall function in oocyte meiosis reinitiation could be resolved if a suitable denuding technique were available. Thus, to facilitate future investigations of the interactions occurring among the follicle wall, the oocyte, and exogenously applied hormones in vitro, we developed a method to obtain brook trout oocytes denuded of somatic cells. A combination of techniques involving light and scanning electron microscopy along with hormone bioassay were employed to compare intact follicles and denuded oocytes in brook trout. Our major objective was to remove layers from the follicle wall of the large ovarian follicles in a stepwise manner.

Materials and Methods

Adult female brook trout weighing approximately 400 g were obtained from the Nova Scotia provincial hatchery at Fraser's Mills. Animals with fully developed ovaries were held at 14 h photophase and 10°C in 500-L flow-through tanks (Living Stream, Frigid Units Inc.). Fish were stunned by a blow to the head, decapitated immediately, and the ovary was quickly dissected and placed in Cortland's solution (Wolf and Quimby 1969).

Our initial attempts to manually remove the follicle wall in normal Cortland's were unsuccessful; this is in contrast with the relative ease with which frog oocytes can be manually defolliculated in a similar medium (Masui 1967; Lessman and Schuetz 1981, 1982; Schuetz and Lessman 1982). Two approaches were taken to remove the wall. The first, based on preincubation of ovarian fragments in Tween 80 (5 mg/L), was later abandoned due to the possibility that the detergent would damage the oocyte membrane. The second approach was based on the use of Ca^{2+}-free Cortland's solution to dissociate the layers of the follicle wall and allow subsequent microdissection of the wall components. This approach was guided by the observation that after manual defolliculation, residual follicle cells could be removed from frog oocytes by treatment with Ca^{2+}-free media (Lessman and Schuetz 1981).

We prepared the follicles and oocytes as follows. After two media rinses, the ovary was incubated overnight in Ca^{2+}-free Cortland's solution at 4°C in the dark. Intact follicles were dissected from other ovarian tissue using watchmaker forceps (Dumont No. 4) and a Nikon SMZ-10 stereomicroscope. To obtain oocytes denuded of follicle wall components, we removed the ovarian epithelium containing blood vessels using watchmaker forceps with highly sharpened tips. It was possible to remove the ovarian epithelium by scoring the surface with one tip of a forceps, producing a slit from which a portion of the oocyte protruded. Using two pairs of forceps, one tip of each inserted between the ovarian epithelium and the adjacent layers, the ovarian epithelium was removed as a single sheet by gradually opening the slit. The theca folliculi was usually removed along with the ovarian epithelium; however, in some cases the theca folliculi also had to be removed with forceps. The follicle cell epithelium was removed by shaking oocytes in Ca^{2+}-free Cortland's solution at 15–20°C for 15 min at 100 oscillations/min.

Intact follicles and follicles in different stages of denudation were prepared for scanning electron microscopy (SEM). They were fixed in 2% gluteraldehyde in Cortland's solution, washed in sodium cacodylate buffer (0.1 mol/L), postfixed in 1% osmium tetroxide, and dehydrated in a graded series of acetone. The preparations were critical point dried from liquid CO_2 (Polaron e-5300 drier), mounted with silver paint onto aluminum stubs, and coated with carbon followed by gold–palladium alloy using a sputter coater (Samsputter, Tousimis Co.). The preparations were then observed with SEM (Nonlab 200, Bausch & Lomb) at 15 kV and the images recorded on TRI-X film (Kodak).

The responsiveness of the intact follicles and denuded oocytes to steroids and pituitary extract was tested after equilibrating in normal Cortland's solution (containing Ca^{2+}) for 60 min. They were dispensed 20 to a well containing 2 mL of Cortland's solution in a multiwell tissue culture dish (Costar 3524). Acetone-dried carp pituitary (Crescent Chem.) was homogenized in Cortland's solution and centrifuged at 2000 × *g* for 15 min. The supernatant was removed and stored as aliquots in sealed glass ampoules at −20°C until required. This stock solution of carp pituitary homogenate (CPH) had a concentration of 10 mg/mL.

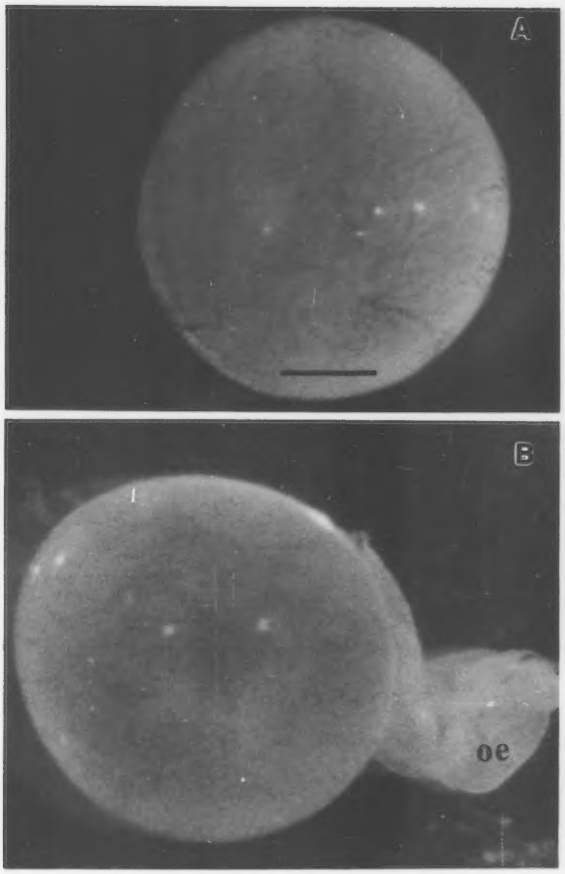


FIG. 1. Light micrographs of brook trout ovarian follicles. (A) Intact follicle with extensive blood vessel network in the ovarian epithelium. Bar = 1 mm. (B) Follicle with ovarian epithelium (oe) removed; note absence of blood vessels on oocyte surface.

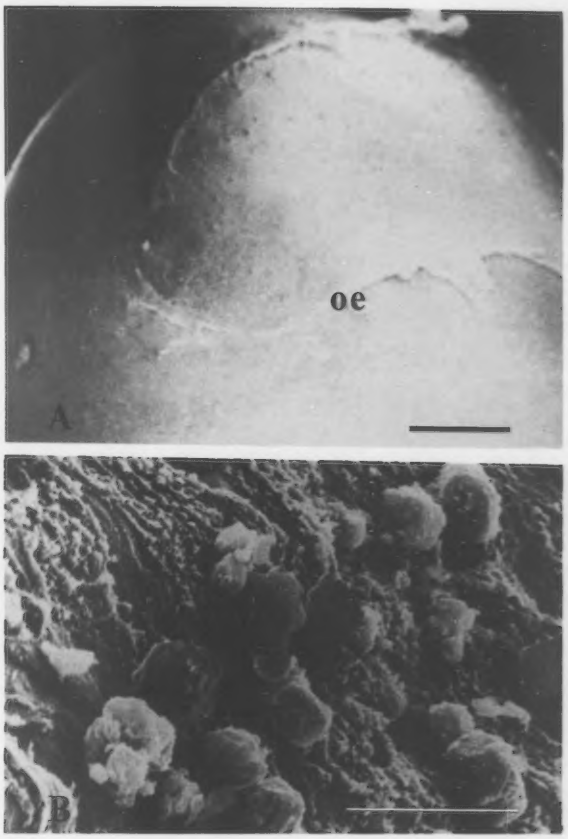


FIG. 2. Scanning electron micrographs of brook trout ovarian follicles. (A) Low magnification view of intact follicle; note ovarian epithelium (oe) which forms ridge at junction with rest of ovary. Bar = 500 μm. (B) High magnification view of intact follicle surface; note complex of cells and fibers. Bar = 10 μm.

Steroid (17α,20β-dihydroxy-4-pregnen-3-one, DHP, Sigma) was dispensed in a steroid vehicle (EtOH – propylene glycol, 1:1). The steroid vehicle final concentration was not greater than 0.1%; negative controls and CPH-treated wells also received the vehicle. After incubation for 72 h at 15°C, 200 μL of 50% acetic acid was added to each well. The acid cleared the yolky cytoplasm and precipitated the nucleoplasm such that the nucleus or germinal vesicle (GV), if present, could be readily observed with transmitted light under a stereo dissecting microscope. Resulting data were analyzed statistically by a test of proportions based on binominals (Downie and Heath 1965).

Results

After Ca^{2+}-free treatment and manual defolliculation, removal of the ovarian epithelium could be verified easily due to the blood vessel content of this tissue (Fig. 1). In SEM, the intact follicle surface has an irregular topography containing fibers and cells (Fig. 2). Following removal of the ovarian epithelium (Fig. 1), the theca folliculi was seen at the light microscope level as wisps of white tissue adhering to the oocyte surface. In the electron micrographs the theca folliculi appeared as a sheet that was easily torn revealing the underlying follicle cell epithelium (Fig. 3). After Ca^{2+}-free treatment (15–20°C, 15 min, 100 oscillations/min), the follicle cell epithelium was removed leaving the chorion denuded of cells (Fig. 4). The surface of the denuded oocyte had an appearance resembling that of naturally ovulated eggs (Brummett et al. 1982; Cherr and Clark 1982; Dumont and Brummett 1980; Hart and Donovan 1983; Kobayashi and Yamamoto 1981; Ohta and Iwamatsu 1983).

Denuded oocytes were induced to undergo meiosis reinitiation as evidenced by germinal vesicle dissolution (GVD) in response to DHP treatment (Table 1). Oocytes were dissected after acetic acid fixation and the presence or absence of the germinal vesicle was determined. The presence of the germinal vesicle was verified cytologically by observing isolated germinal vesicles containing distinctive nucleoli with phase contrast optics (Fig. 5). The denuding process augmented steroid responsiveness rather than decreasing it, suggesting that the denuding technique did not have any deleterious effect on the oocytes. Carp pituitary homogenate elicited significant GVD in follicle-enclosed oocytes, but was without effect on denuded oocytes.

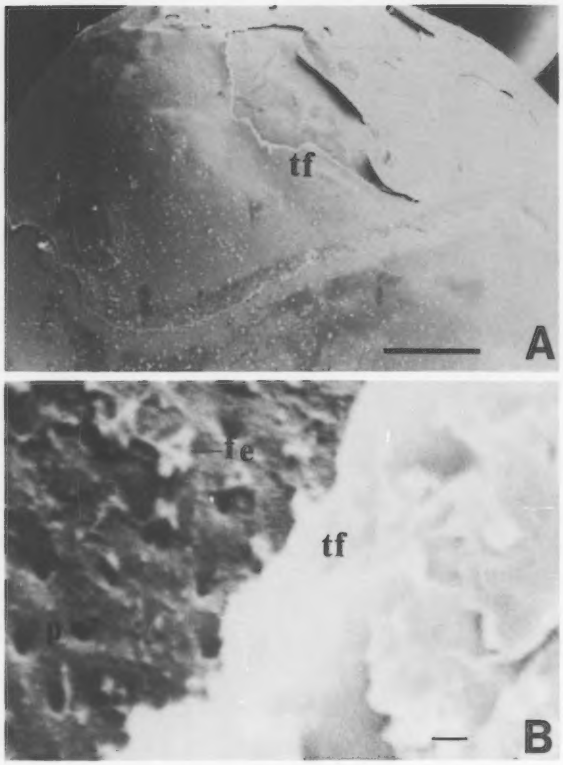


FIG. 3. Scanning electron micrographs of brook trout ovarian follicles with ovarian epithelium removed. (A) Low magnification view showing theca folliculi (tf) which is torn exposing underlying follicle cell epithelium. Bar = 500 μm. (B) High magnification view showing detail of theca folliculi and underlying follicle cell epithelium (fe) made up of individual follicle cells; pores (p) of the chorion are indicated. Bar = 1 μm.

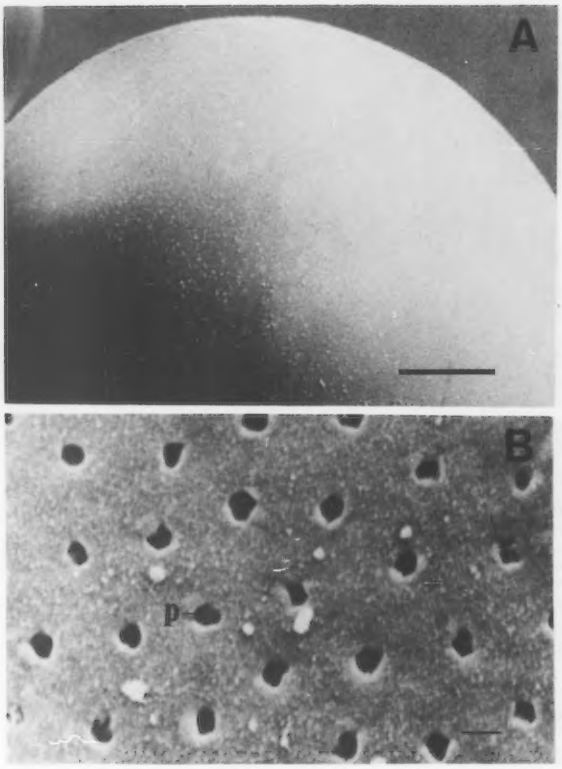


FIG. 4. Scanning electron micrographs of denuded oocytes of brook trout. (A) Low magnification view of oocyte after theca folliculi and follicle cell epithelium have been removed by Ca^{2+}-free Cortland's solution. Bar = 500 μm. (B) High magnification view of denuded oocyte surface; pores (p) of the chorion are visible while somatic cells are absent. Bar = 1 μm.

Discussion

The technique of oocyte denudation in amphibians has resulted in significant progress in understanding their follicle function. These same processes in fish, however, are less understood. Some work with denuded or partially denuded fish oocytes has been reported (Jalabert 1976; Iwamatsu and Katoh 1978; Iwamatsu 1980). Unfortunately, none of these studies included SEM of oocyte surfaces. In spite of these pioneering studies, the role of the follicle wall in fish oocyte meiosis remains unclear. The confusion may be due to a lack of clearly defined morphological criteria for the denuded fish oocyte. It might be helpful to define the state of denuding in the fish follicle (see Fig. 6) using criteria published for *Rana pipiens* (Schuetz and Lessman 1982).

Support for the view that the follicle wall is involved in steroid synthesis in lower vertebrates is substantial (Masui and Clarke 1979). In the salmon ovary, cells of the theca (theca folliculi) and granulosa (follicle cell) layers have been reported to be the major sites of steroid synthesis (Nagahama et al. 1982; Kagawa et al. 1982). Nevertheless, a clear understanding of the interaction of various components of the follicle wall with gonadotropin and the relationship to steroid production is not extant. Cells in the thecal layer of preovulatory follicles of

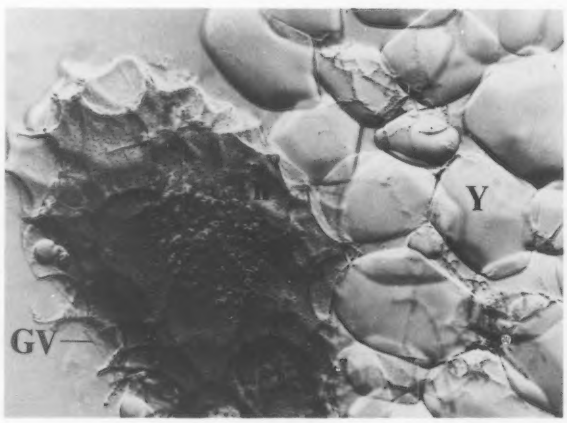


FIG. 5. Isolated germinal vesicle (GV) containing numerous nucleoli (n) from an acetic acid fixed brook trout oocyte. Large clear structures to the right of the germinal vesicle are yolk globules (Y). Field photographed at 200 × using phase contrast optics.

TABLE 1. Effect of follicle wall removal on hormone-induced germinal vesicle dissolution (GVD) during meiosis reinitiation in oocytes of the brook trout, *Salvelinus fontinalis*. Data are numbers of oocytes with nuclear dissolution/total number tested (% GVD) based on 10–20 oocytes or follicles per treatment per female ($N = 5$ or 6); values followed by a different letter are significantly different at $P < 0.05$.

Treatment	Intact follicles	Denuded oocytes
Carp pituitary (100 μg/mL)	44/99 (44.4%) a	24/89 (27.0%) c
17α,20β-dihydroxy-4-pregnen-3-one (1 μg/mL)	61/124 (49.2%) a	70/111 (63.1%) b
Control (vehicle only)	31/127 (24.4%) c	30/115 (26.1%) c

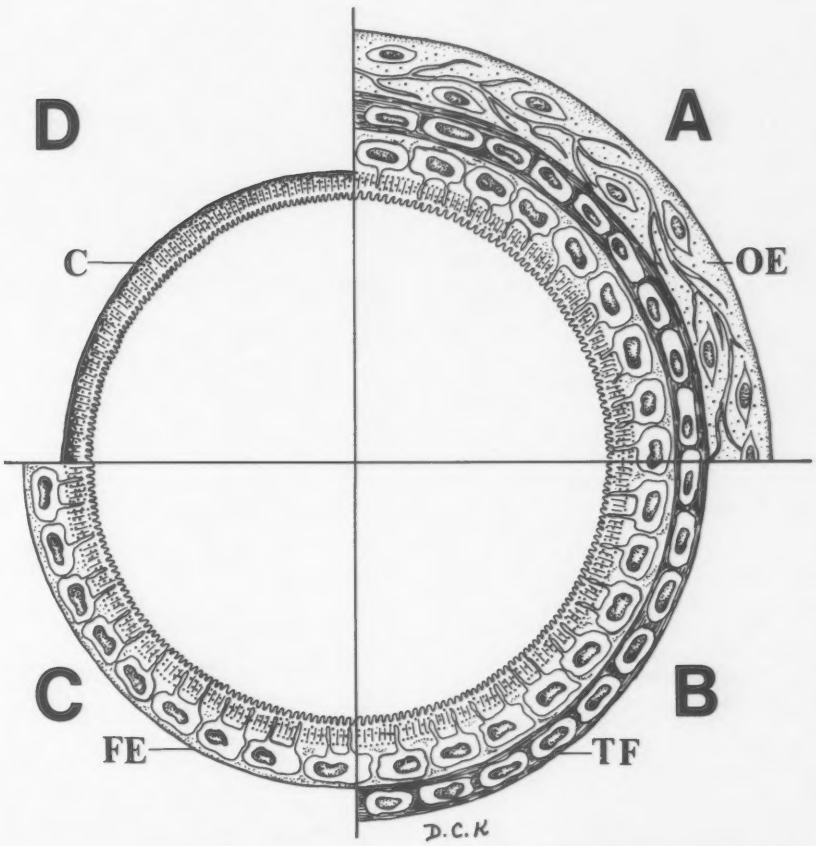


FIG. 6. Diagrammatic summary of stepwise removal of the ovarian follicle wall components of brook trout (oocyte and wall components not drawn to scale). (A) Intact follicle (type I) with ovarian epithelium (OE) containing blood vessels. (B) Follicle (type II) with ovarian epithelium removed exposing theca folliculi (TF). (C) Follicle (type III) with theca folliculi removed. This layer is often removed along with the ovarian epithelium. The layer exposed is the follicle cell epithelium (FE). (D) Oocyte (type IV follicle) denuded of follicle cell epithelium exposing the chorion (C).

goldfish show 3β-hydroxy-Δ^5-steroid dehydrogenase activity (Nagahama et al. 1976), which is indicative of steroid synthesis. In contrast with intact follicles, denuded oocytes of *Rana pipiens* do not synthesize progesterone in response to gonadotropin (Lessman and Schuetz 1982; Schuetz and Lessman 1982). Furthermore, the progesterone synthesis inhibitor, cyanoketone, inhibited GVD induction by gonadotropin and insulin in *Rana* follicles (Lessman and Schuetz 1981). Conversion of pregnenolone to progesterone was reported by Schwartz and Ziegler (1979) in isolated follicle cells from *Rana* follicles; however, denuded oocytes were not found to carry out this conversion.

We have put forth a method for the removal of the follicle wall components of the brook trout ovarian follicle (Fig. 6). The technique involved preincubation of ovaries in vitro in Ca^{2+}-

free Cortland's solution at 4°C for at least 10 h, followed by microdissection of the ovarian epithelium and theca folliculi. The follicle cell epithelium is finally removed by shaking the oocytes in Ca^{2+}-free Cortland's solution. This process results in denuded oocytes which lack somatic cells as evidenced by SEM. Further, the denuded oocytes remain in prophase I, since they possess a germinal vesicle, and respond to steroids by undergoing meiotic reinitiation. However, the denuded oocytes do not undergo GVD after gonadotropin treatment. Although exposure to Ca^{2+}-free media is somewhat prolonged in our procedure, the oocytes nevertheless appear morphologically normal and respond to steroids. Very likely the Ca^{2+} depletion is not as extensive as in other published procedures which use EDTA in addition to Ca^{2+}-free media (Schatz and Ziegler 1979). Exposure of *Rana* oocytes to Ca^{2+}-free media temporarily alters the electrophysiology of the denuded oocyte (Lessman and Marshall 1984); however, this change could be reversed by equilibration in Ringer's containing Ca^{2+}. Thus the procedure might be preferable to those in which enzymes are used to remove follicular components, since changes induced by enzymes would probably be irreversible. Attempts to denude goldfish (*Carassius auratus*) oocytes in our laboratory using collagenase, trypsin, or pronase have been unsuccessful (C. A. Lessman, unpubl. data).

We observed some spontaneous maturation which could be attributable to the Ca^{2+}-free treatment; however, in other studies we have found a low, yet consistent level of spontaneous maturation without Ca^{2+}-free treatment in fish oocytes cultured in vitro (Lessman and Kavumpurath 1984; Lessman 1985). Possibly, spontaneous maturation, as well as responsiveness to progestogens, fluctuates seasonally and may also be related to varying amounts of follicle atresia. Our procedure includes verification of germinal vesicle identity by cytological means (Fig. 5). Other reports, in which germinal vesicle identity was not documented, may differ from our findings in regard to spontaneous maturation and progestogen responsiveness.

Investigations with brook trout intact follicles in vitro have demonstrated a hierarchy of GVD induction by different exogenous steroids (Duffey and Goetz 1980; Goetz and Bergman 1978; Theofan and Goetz 1983). The technique of denuding brook trout oocytes will allow important future studies probing the interactions occurring among hormones (both protein and steroid), follicle wall components, and the oocyte. An important feature of this system is the inherent bioassay for GVD which readily allows assessment of hormone–cell interaction in light of biological activity.

Acknowledgements

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada. We thank Deb Kennedy for artwork, Barbara Pluta and Debra Henke for technical help, Mary Murphy for manuscript preparation, and Anne Louise MacDonald for animal care. We acknowledge the use of SEM facilities provided by Dr. Brian Hall, and we are grateful for informative discussions with Dr. Yoshio Masui.

References

BRUMMETT, A. R., J. N. DUMONT, AND J. R. LARKIN. 1982. The ovary of *Fundulus heteroclitus*. J. Morphol. 173: 1–16.

CHERR, G. N., AND W. H. CLARK JR. 1982. Fine structure of the envelope and micropyles in the eggs of the white sturgeon, *Acipenser transmontanus* Richardson. Dev. Growth Differ. 24: 341–352.

DOWNIE, N. M., AND R. W. HEATH. 1965. Basic statistical methods. 2nd ed. Harper and Row, New York, NY. p. 146–153.

DUFFEY, R. J., AND F. W. GOETZ. 1980. The *in vitro* effects of 17α-hydroxy-20β-dihydroprogesterone on germinal vesicle breakdown in brook trout (*Salvelinus fontinalis*) oocytes. Gen. Comp. Endocrinol. 41: 563–565.

DUMONT, J. N., AND A. R. BRUMMETT. 1980. The vitelline envelope, chorion, and micropyle of *Fundulus heteroclitus* eggs. Gamete Res. 3: 25–44.

FLUGEL, H. 1967. Licht- und elektronemikroskopische untersuchungen an oozyten und eiern einiger knochenfische. Z. Zellforsch. 83: 82–116.

GOETZ, F. W., AND H. L. BERGMAN. 1978. The effects of steroids on final maturation and ovulation of oocytes from brook trout *Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). Biol. Reprod. 18: 293–298.

GURAYA, S. S. 1978. Maturation of the follicular wall of nonmammalian vertebrates, p. 261–329. *In* R. E. Jones [ed.] The vertebrate ovary Plenum Press, New York, NY.

HART, N. H., AND M. DONOVAN. 1983. Fine structure of the chorion and site of sperm entry in the egg of *Brachydanio*. J. Exp. Zool. 227: 277–296.

IWAMATSU, T. 1980. Studies on oocyte maturation of the medaka, *Oryzias latipes*. VIII. Role of follicular constituents in gonadotropin- and steroid-induced maturation of oocytes *in vitro*. J. Exp. Zool. 211: 231–239.

IWAMATSU, T. AND T. KATOH. 1978. Maturation *in vitro* of oocytes of the loach, *Misgurnus anguillicaudatus* by steroid hormones and gonadotropins. Annot. Zool. Jpn. 51: 79–89.

JALABERT, B. 1976. In vitro oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*), and goldfish (*Carassius auratus*). J. Fish. Res. Board Can. 33: 974–988.

KAGAWA, H., G. YOUNG, S. ADACHI, AND Y. NAGAHAMA. 1982. Estradiol-17β production in amago salmon (*Oncorhynchus rhodurus*) ovarian follicles: role of the thecal and granulosa cells. Gen. Comp. Endocrinol. 47: 440–448.

KOBAYASHI, W., AND T. S. YAMAMOTO. 1981. Fine structure of the micropylar apparatus of the chum salmon egg, with a discussion of the mechanism for blocking polyspermy. J. Exp. Zool. 217: 265–275.

LESSMAN, C. A. 1985. Effect of insulin on meiosis reinitation induced in vitro by three progestogens in oocytes of the goldfish (*Carassius auratus*). Dev. Biol. 107: 259–263.

LESSMAN, C. A., AND S. KAVUMPURATH. 1984. Cytological analysis of nuclear migration and dissolution during steroid-induced meiotic maturation *in vitro* of follicle-enclosed oocytes of goldfish (*Carassius auratus*) Gamete Res. 10: 21–29.

LESSMAN, C. A., AND W. S. MARSHALL. 1984. Electrophysiology of *in vitro* insulin- and progesterone-induced reinitiation of oocyte meiosis in *Rana pipiens*. J. Exp. Zool. 231: 257–266.

LESSMAN, C. A., AND A. W. SCHUETZ. 1981. Role of follicle wall in insulin induced meiosis reinitiation in *Rana pipiens* oocytes. Am. J. Physiol. 241: E51–E56.

1982. Insulin induction of meiosis in *Rana pipiens* oocytes: relation to endogenous progesterone. Gamete Res. 6: 95–106.

MASUI, Y. 1967. Relative roles of the pituitary, follicle cells, and progesterone in the induction of oocyte maturation in *Rana pipiens*. J. Exp. Zool. 166: 365–376.

MASUI, Y., AND H. J. CLARKE. 1979. Oocyte maturation. Int. Rev. Cytol. 57: 185–282.

NAGAHAMA, Y., K. CHAN, AND W. S. HOAR. 1976. Histochemistry and ultrastructure of pre- and post-ovulatory follicles in the ovary of the goldfish, *Carassius auratus*. Can. J. Zool. 54: 1128–1139.

NAGAHAMA, Y., H. KAGAWA, AND G. YOUNG. 1982. Cellular sources of sex steroids in teleost gonads. Can. J. Fish. Aquat. Sci. 39: 56–64.

OHTA, T., AND T. IWAMATSU. 1983. Electron microscopic observations on sperm entry into eggs of the rose bitterling, *Rhodeus ocellatus*. J. Exp. Zool. 227: 109–119.

SCHATZ, F., AND D. ZIEGLER. 1979. The role of follicle cells in *Rana pipiens* oocyte maturation induced by delta 5-pregnenolone. Dev. Biol. 73: 59–67.

SCHUETZ, A. W., AND C. A. LESSMAN. 1982. Role of the surface epithelium and follicle wall in ovulation and progesterone production by frog (*Rana pipiens*) follicles. Differentiation 22: 79–84.

THEOFAN, G., AND F. W. GOETZ. 1983. The *in vitro* synthesis of final maturational steroids by ovaries of brook trout (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). Gen. Comp. Endocrinol. 51: 84–95.

WOLF, K., AND M. C. QUIMBY. 1969. Fish cell and tissue culture, p. 293–312. *In* W. S. Hoar and D. J. Randall [ed.] Fish. physiology. Vol. 3. Academic Press, New York, NY.

Changes to Stock Reduction Analysis Indicated by Schnute's General Theory

Daniel K. Kimura

National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northwest and Alaska Fisheries Center, 7600 Sand Point Way N.E., BIN C15700, Building 4, Seattle, WA 98115-6349, USA

Kimura, D. K. 1985. Changes to Stock Reduction Analysis indicated by Schnute's general theory. Can. J. Fish. Aquat. Sci. 42: 2059–2060.

Recently, J. Schnute generalized the R. B. Deriso age-structured model by using a more general growth curve to derive a more general form of Deriso's delay-difference equation. I have outlined changes in the Stock Reduction Analysis (SRA) model that are required to take advantage of Schnute's more general approach. The proposed changes in the equations do not affect the way SRA is applied, but should reduce bias in the model.

Récemment, J. Schnute généralisait le modèle structuré par âge établi par R. B. Deriso en utilisant une courbe de croissance plus générale pour en tirer une formule elle aussi plus générale de l'équation à variables retardées de Deriso. J'ai indiqué les changements apportés au modèle d'analyse des stocks avec paramètres réduits (ASPR) qui sont nécessaires pour tirer profit de l'approche plus générale de Schnute. Les changements proposés dans les équations ne modifient pas la façon dont l'ASPR est appliquée, mais devraient réduire les inexactitudes dans le modèle.

Received April 16, 1985
Accepted August 8, 1985
(J8193)

Reçu le 16 avril 1985
Accepté le 8 août 1985

Schnute (1985) generalized the Deriso (1980) model by using a more general growth curve to derive a more general form of the delay-difference equation. While Schnute's modifications to the Deriso model appear slight, the new model can lead to different results. The purpose of this note is to present changes to Stock Reduction Analysis (SRA) indicated by Schnute's more general model. I will follow, as much as possible, the notation used by Kimura et al. (1984).

Because the form of the growth model determines the form of the delay-difference equation, it is important that the proper growth curve be used with a delay-difference equation. For example, parameters estimated using Schnute's form of the growth model should *not* be applied to Deriso's form of the delay-difference equation.

For a given age-at-recruitment, k, Schnute (1985, equation 1.14) proposed the growth model

$$(1)\quad W_{k+j} = \omega_{k-1} + (\omega_k - \omega_{k-1})(1 - \rho^{1+j})/(1 - \rho) \quad \text{for } j \geq 0$$

where ω_{k-1}, ω_k, and ρ are unknown parameters that can be estimated using least squares, and W_{k+j} is the average weight of $k + j$ yr olds. Note that ω_{k-1} and ω_k should approximate W_{k-1} and W_k. Schnute shows that this growth model is equivalent to the von Bertalanffy growth curve. For the sake of simplicity, Deriso (1980) assumed $\omega_{k-1} = 0$. As the example given by Schnute shows, this simplified model may not always be adequate for fitting weight-at-age data.

Schnute (1985) goes on to derive the delay-difference equation based on equation 1:

$$(2)\quad B_t = (1 + \rho)s_{t-1}B_{t-1} - \rho s_{t-1}s_{t-2}B_{t-2} + R_t - \rho(W_{k-1}/W_k)s_{t-1}R_{t-1}.$$

Except for the final term, equation 2 is identical to Deriso's form of the delay-difference equation.

To perform SRA in a manner consistent with Schnute's generalization of Deriso's model, I suggest estimating ω_{k-1}, ω_k, and ρ from equation 1, and then applying these estimates to equation 2, where $\omega = W_{k-1}/W_k$ can be estimated by the ratio of least squares estimates ($\hat{\omega} = \hat{\omega}_{k-1}/\hat{\omega}_k$).

There are four equations in SRA that need to be changed to accommodate Schnute's model. The equations below replace the primed equations in Kimura et al. (1984):

$$(3)\ (2')\quad B_t = (1 + \rho)s_{t-1}B_{t-1} - \rho s_{t-1}s_{t-2}B_{t-2} + R_t - \rho\omega s_{t-1}R_{t-1}$$

$$(4)\ (4')\quad B_2 = (1 + \rho)s_1B_1 - \rho s_1 s_0 B_0 + R_2 - \rho\omega s_1 R_1$$

$$(5)\ (7')\quad R_1 = B_1\{[1 - \exp(-M)] + \rho[\exp(-2M) - \exp(-M)]\}/[1 - \rho\omega\exp(-M)]$$

$$(6)\ (8')\quad B = \{[R_1(1 - \rho\omega s)/B_1']/[1 + \rho s^2 - (1 + \rho)s]\}^{\frac{1}{1-r}}.$$

With these four changes, SRA can be performed using Schnute's more general model.

For convenience in checking computer programs, I provide a limited reanalysis, using equations 3–6, of the application of SRA to Pacific ocean perch (*Sebastes alutus*) given by Kimura et al. (1984). Fitting the Pacific ocean perch weight-at-age data to equation 1, I estimate

$$\hat{\omega}_{k-1} = 0.464 \quad \hat{\omega}_k = 0.566 \quad \hat{\rho} = 0.931$$

and therefore

$$\hat{\omega} = \hat{\omega}_{k-1}/\hat{\omega}_k = 0.82.$$

Assuming B_1 is a virgin biomass, recruitment to the virgin biomass can be estimated using the "expected recruitment line" (equation 5). Unique solutions to the SRA equations can then be derived from assumed values of B_1 and r, where r is the exponent in the Cushing recruitment model (Kimura et al. 1984). Two such possible solutions to the new SRA equations are

$B_1 = 1\,100\,000$ t $\quad R_1 = 88\,774$ t $\quad r = 0$

$P = 0.303 \quad$ MSY = 62 146 t (assuming $F = M$)

and

$B_1 = 1\,100\,000$ t $\quad R_1 = 88\,774$ t $\quad r = 0.5$

$P = 0.154 \quad$ MSY = 28 175 t

where $P = B_{n+1}/B_1$ is the estimated ratio between final biomass and virgin biomass and MSY is an estimate of maximum sustainable yield.

For given values of B_1 and r, the present analysis gives a slightly larger value of recruitment (R_1), and moderately lower values of P and MSY, than the previous analysis by Kimura et al. (1984). One explanation might be that the original analysis used a growth curve that overestimated the weight of the younger, more abundant fish and therefore overestimated MSY.

However, it may not be wise to make general statements based on these differences because they depend on parameter values (B_1, r, ρ, ω) and the way in which SRA was applied. For example, if the SRA equations were constrained by fixing P instead of B_1, the estimates of MSY using Schnute's form of the delay-difference equation would be more similar to those in the previous analysis.

Acknowledgments

I thank Dr. Jon Schnute of the Pacific Station for reviewing this manuscript.

References

DERISO, R. B. 1980. Harvesting strategies and parameter estimation for an age-structured model. Can. J. Fish. Aquat. Sci. 37: 268–282.

KIMURA, D. K., J. W. BALSIGER, AND D. H. ITO. 1984. Generalized Stock Reduction Analysis. Can. J. Fish. Aquat. Sci. 41: 1325–1333.

SCHNUTE, J. 1985. A general theory for analysis of catch and effort data. Can. J. Fish. Aquat. Sci. 42: 414–429.

PERSPECTIVES

Seaweeds, Sea Urchins, and Lobsters: A Reappraisal

Robert J. Miller

Halifax Fisheries Research Laboratory, Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S. B3J 2S7

Miller, R. J. 1985. Seaweeds, sea urchins, and lobsters: a reappraisal. Can. J. Fish. Aquat. Sci. 42: 2061–2072.

A popular hypothesis, that on the south coast of Nova Scotia the green sea urchin (*Strongylocentrotus droebachiensis*) has been released from control by predators, is critically reviewed. Predator control is given the functional definition of limiting sea urchin grazing sufficient to permit abundant growth of seaweed. Of the predators identified, the American lobster (*Homarus americanus*) is the least likely to have been important in sea urchin control in recent decades. Feeding rates, stomach contents, and field biomass are available for this species. A smaller amount of data available for the rock crab (*Cancer irroratus*) and benthic feeding fish points to the conclusion that they likewise cannot control urchin populations. Among other temperate sea urchin species the case is strongest for sea otter control of *Strongylocentrotus* sp. in some areas of the west coast of North America. The formation of sea urchin grazing fronts, a usual step in the destruction of seaweed beds, has been explained as a behavioral response to predators. This may in fact be merely a response to the location of food. Evidence for suspected enhancement of lobster production by seaweeds is equivocal, and comparative field data collected in and out of seaweed beds (e.g. lobster abundance, food, growth, and survival) have not been published.

L'auteur vérifie une hypothèse populaire selon laquelle le nombre d'oursins verts (*Strongylocentrotus droebachiensis*) présents sur le littoral sud de la Nouvelle-Écosse n'est plus contrôlé par les prédateurs. Du contrôle par les prédateurs, il donne la définition fonctionnelle suivante : le niveau de limitation du broutage des algues marines par les oursins suffisant pour permettre une croissance abondante des algues. Parmi les prédateurs identifiés, le homard (*Homarus americanus*) est probablement le moins important pour ce qui est du contrôle du nombre d'oursins au cours des dernières décennies. Des données sur les taux d'alimentation, les contenus stomacaux et la biomasse en milieu sauvage de l'oursin sont disponibles. Une plus faible quantité de données sur le crabe commun (*Cancer irroratus*) et les poissons benthivores laisse supposer qu'ils ne peuvent pas contrôler les populations d'oursin. Pour ce qui est des autres espèces d'oursin peuplant les eaux tempérées, les données indiquent que la loutre marine est un important prédateur de *Strongylocentrotus* sp. dans certaines régions de la côte ouest de l'Amérique du Nord. Le regroupement des oursins en fronts de broutage, étape habituelle dans la destruction des bancs d'algues, est considéré comme une réaction comportementale aux prédateurs. En fait, ce n'est peut-être qu'une réaction à la détermination de l'emplacement de la nourriture. Le fait que la présence de bancs d'algues accroisse la production de homard est ambigu. Des données comparatives recueillies à l'intérieur et à l'extérieur des bancs d'algues, par ex. sur le nombre de homards, la nourriture, la croissance et la survie, n'ont pas encore été publiées.

Received March 7, 1985
Accepted July 31, 1985
(J8140)

Reçu le 7 mars 1985
Accepté le 31 juillet 1985

Reasonable hypotheses that the green sea urchin (*Strongylocentrotus droebachiensis*) on Nova Scotia shores has been released from control by predators, especially the American lobster (*Homarus americanus*), have been offered and repeated often. With little difficulty I located 26 papers published from 1972 through 1983 referring to these hypotheses, and the authors of only three of these expressed disagreement. At least some fishermen from a century ago would have also agreed. A Royal Commission (Anonymous 1887, p. 48–49) received the following testimony concerning an area east of Halifax: "... fishermen in Shoal Bay say that sea vermin, sea fleas and such like have destroyed the seaweed and left the rock bare. The corollary is that the lobsters, scavengers of the water, from being so reduced in numbers, have lost balance over the sea vermin. ..." However, from a review of the literature and a small amount of new data, I will argue that the evidence does not support these popular hypotheses. Also, the literature on other species of temperate sea urchins makes few strong cases for predator control. Some of this evidence was considered by Choat (1982), who discovered that although recent literature argued that predatory fish were major determinants in structuring coastal temperate benthic communities, most of the associated data did not support this conclusion.

Published claims that the presence of seaweed beds enhance lobster production are also critically reviewed. Although supported by logical arguments, a cause–effect relationship has not

been established and important measurements to test the idea have not been made.

For the following discussion, "predator control" is defined as a limit to sea urchin grazing sufficient to permit growth of abundant seaweed on habitat suitable for seaweed growth. This functional definition in terms of impact on seaweed is preferred to the more usual measure of control, fewer prey in the presence of predators. Seaweed is frequently the community component of interest because of its structural dominance and because of its very considerable production. Also, urchin impact on seaweed is often all or none, i.e. urchins either maintain rocks nearly free of seaweed or they have little effect. The green sea urchin is able to maintain its habitat nearly free of seaweed probably because of its adaptability to a wide range of food quality and quantity (Johnson and Mann 1982; Thompson 1983). Moderately high densities survive on mostly barren rock, feeding opportunistically on meagre rations of ephemeral seaweed, drift seaweed, calcareous algae, and microalgae; or they are able to take relatively large rations. The above definition of control includes continued maintenance of abundant seaweed as well as predator-effected change from urchin-dominated barrens to abundant seaweed.

Predator Control of the Green Sea Urchin

History

The expansion of rock habitat dominated by green sea urchins at the expense of seaweed cover is well documented for St. Margarets Bay, Nova Scotia (Breen and Mann 1976; Chapman 1981). Because seaweeds (attached macrophytes except calcareous forms) returned when sea urchins were removed, both the above investigations showed convincingly that urchins were the direct cause of seaweed disappearance.

The expansion of sea urchin habitat was first attributed by Mann and Breen (1972) to the release of urchins from predation. These authors hypothesized that the American lobster was a key predator in this community and that reducing its density by fishing had permitted sea urchins to increase. Using a simulation model, Breen (1974) and Breen and Mann (1976) tested and supported the hypothesis that lobster was a key predator and that it could control the urchin population over a range of community states. They also concluded that the rock crab (*Cancer irroratus*) was a significant but less important predator, and that the Atlantic wolffish (*Anarhichas lupus*) was an unimportant predator contributing to urchin control. Later, based on increased estimates of crab abundance (Drummond-Davis 1978) and decreased estimates of lobster preference for sea urchins as prey (Evans and Mann 1977), the model was rerun and predicted that the importance of crab predation approached that of lobster (D.A. Coleman, unpubl. data, quoted in Breen 1980). In addition to the above results the Breen and Mann (1976) study was important for providing many observations and hypotheses that were the bases of most subsequent studies conducted in Nova Scotia on sea urchin–predator interactions.

Bernstein et al. (1981) introduced a more complex scenario for predator control. In healthy seaweed beds sea urchins in low density remain hidden under rocks to avoid predators. If predation pressure is reduced, e.g. by lobster removal, sea urchins increase in density and come out of hiding in search of food. Remaining decapod predators then cause urchins to form defensive aggregations which move en masse to graze holes in the bed until eventually it is eliminated. However, certain fish species that can prey on urchin aggregations and that are abundant in summer may cause a seasonal breakup, reducing grazing. Later, Bernstein et al. (1983) conducted field experiments in cages and observed the aggregation response of urchins with and without predators present. They supported the above scenario but added complexity to the aggregation response, e.g. urchins respond differently to crabs and lobsters in and out of seaweed beds and at high and low densities.

In summarizing the discussion from a workshop on seaweed–herbivore–carnivore interactions, Pringle et al. (1980) listed several probable predators of each sea urchin life history stage. They concluded that over the last few decades, increased fishing pressure on several urchin predators as well as unknown variation in abundance of predators not fished prevented ranking them in order of abundance. They also reviewed the evidence that environmental factors and overfishing caused recent declines in lobster landings.

In response to the above conclusions, Wharton and Mann (1981) agreed that urchins had a suite of predators, but emphasized the importance of lobsters and fish predators. They agreed with Pringle et al. (1980) that overfishing and environmental causes could have been important in initial lobster decline. A major contribution of Wharton and Mann was documenting the widespread occurrence of urchin-dominated habitat. Mann (1977, 1982) has also summarized his and co-workers' studies on predator control of sea urchins.

Lobster as Predator

Data from experimental studies on the feeding rates of lobsters and stomach analysis of field-caught lobsters (Table 1) led to the conclusion that they kill the equivalent of their body weight in sea urchins per year or, given the uncertainties in calculations, 0.5–2 times their body weight per year. When offered a choice of sea urchins and more desirable prey, i.e. crabs and mussels (a choice between urchins and undesirable prey species would not be a fair measure of lobster predation on urchins), lobsters killed about 0.4% of their body weight per day in urchins when measured near maximum annual temperatures (Table 1, Ref. 4, 5, and 7). Adjusting for the annual temperature regime in southern Nova Scotia (Trites 1982) would halve this value (Table 1, Ref. 1 and 4; assumed maximum feeding, one-half maximum, and no feeding, each for 4 mo) resulting in a predation rate of 0.7 g sea urchins·g lobster^{-1}·yr^{-1} (0.002 × 365).

A second estimate is derived from the assumptions that lobster total food consumption is 1.5% of body weight per day, that sea urchins comprise only 10% of the lobster diet, and that lobsters consume one third of the weight of the sea urchins they kill. Reference 6 reported a diurnal range of gut fullness for field-caught lobsters of 0.5–1.5%. Reference 9 gave a daily consumption of 4% body weight per day (adjusted for fluid loss of sea urchin prey) when offered a choice of prey. However, because these 1-d measurements were taken after 5 d of starvation, this is arbitrarily reduced to 2% body weight per day. Weighting these two estimates equally for 1.5% body weight per day, and multiplying by 0.1, the fraction of stomach contents of field-caught lobsters that is sea urchins when sea urchins are the most or nearly most abundant prey in the habitat (Table 1, Ref. 3, 6, and 8), gives 0.15% body weight in sea urchins consumed per day. Because lobsters consume only one third of the weight of urchins killed (Table 1, Ref. 9 and 10), the 0.15% is increased to 0.45%, or 1.6 g sea urchin·g lobster^{-1}·yr^{-1}. These calculations are summarized as follows:

TABLE 1. References used to estimate lobster predation on sea urchins. Weights are in live or wet weight.

Reference number	Location	Number of lobsters	Temperature (°C)	Results	Source
1	Laboratory and Bay of Fundy	—	Experimental variable	Lobster locomoter activity increased from 2 to 10°C but was constant 10–20°C; lobster catchability in the field increased from 3 to 11°C	McLeese and Wilder 1958
2	Laboratory	2	Nov. to June: approx. −1 to 7	Lobsters killed 1.2% body weight/day when fed only sea urchins	Himmelman and Steele 1971
3	Northeast Newfoundland	288	−1 to 15	Sea urchins were 7.1% by volume of lobster stomach contents	Ennis 1973
4	St. Margarets Bay, caging experiments	5	July to Sept.: approx. 9–16	Lobsters killed 0.4% body weight/day of sea urchin when offered sea urchins and green crabs	Breen 1974
		5	5–15	Lobsters killed 2–11% body weight/day of sea urchins when offered sea urchins only, densities 0.5 or 1.0 $kg \cdot m^{-2}$	
		5	4	Lobsters did not feed at this temperature	
5	Laboratory	21	12	Lobsters killed 0.5% body weight/day of sea urchins when offered sea urchins and rock crabs	Evans and Mann 1977
6	Southern Gulf of St. Lawrence	471	Autumn	Sea urchins were 7.2% of volume of lobster stomach contents	Scarratt 1980, fig. 3
		541	Spring and autumn	Sea urchins were 19% of volume of lobster stomach contents	Tables 5 and 6
		170	7	Total weight of lobster stomach contents varied diurnally from 0.5 to 1.5% of body weight	
7	Laboratory	15	12.5	Lobsters killed 4.1% body weight/day when offered only sea urchins (corrected for lobsters that did not feed on sea urchins)	Elner 1980a
		4	12.5	Lobsters killed 0.3% body weight/day of sea urchins when offered sea urchins and blue mussels (corrected for lobsters that did not feed on sea urchins)	
8	Southeast Newfoundland	103	Summer and autumn	Sea urchins were 6.6% by volume of lobster stomach contents	Carter and Steele 1982a
9	Laboratory	?	8	Lobsters consumed a maximum of 4% body weight/day when offered four prey species simultaneously	Carter and Steele 1982b
		?	8	22% of live weight of sea urchin consumed by lobster, adjusted for fluid loss	
10	Laboratory	2	12.5	44% of live weight of sea urchins consumed by lobster, adjusted for fluid loss ($n = 7$)	L. V. Colpitts and R. W. Elner, unpubl. data

$$1.6\,\text{g urchins eaten} \cdot \text{g lobster}^{-1} \cdot \text{yr}^{-1}$$
$$= 0.015\,\text{g food eaten} \cdot \text{g lobster}^{-1} \cdot \text{d}^{-1}$$
$$\times 0.1\,\text{g urchin in stomach} \cdot \text{g food in stomach}^{-1}$$
$$\times 3\,\text{g urchin killed} \cdot (3\,\text{g live} - 1.2\,\text{g uneaten} - 0.8\,\text{g fluid})^{-1}$$
$$\times 365\,\text{d} \cdot \text{yr}^{-1}.$$

Where necessary, lobster carapace length (L) was converted to animal live weight (W) by (Thomas 1973)

$$W(\text{g}) = 0.00168 L(\text{mm})^{2.828}$$

and sea urchin diameter was converted to animal live weight by (Miller and Mann 1973)

$$W(\text{g}) = 0.0008 D(\text{mm})^{2.86}.$$

Lobster biomass density, another statistic needed to evaluate

TABLE 2. Field biomass measurements in live weight for *Homarus americanus*.

Location	Month	Depth (m)	Bottom type	Area sampled (m^2)[a]	Lobster biomass ($g \cdot m^{-2}$)	Source
Southern Gulf of St. Lawrence	June	<5	Irish moss beds; flat bedrock ledges	2 900	0.4	Scarratt 1973a
	July	<5	medium rough	900	3.9	
	May–Aug.	<5	boulder strewn	6 300	4.1	
Southern Gulf of St. Lawrence	Spring and fall	~17	Rocky	753[b]	12.6	Scarratt 1968
Southern Gulf of St. Lawrence	July–Sept.	~17	Man-made boulder reef	10 020[c]	11.0	Scarratt 1973b
Nova Scotia Atlantic coast	Aug.–Oct.	3–9	Boulders on sand and gravel	6 700[d]	5.4	Bernstein and Campbell 1983
Nova Scotia Atlantic coast	Sept.	4–8	Kelp bed; boulders on gravel	600	12.8	R. J. Miller, unpubl. data
		4–8	barrens; boulders on gravel	600	9.8	
Nova Scotia Atlantic coast	Year-round	5–12	Boulder and cobble on sand and clay	~360[e]	9.2	Elner and Hamet 1984
Maine						
Stn. 1	Year-round	6–12	Boulders and rocks	13 500[f]	9.0	Cooper et al. 1975
Stn. 2		6–12	on bedrock	13 500[f]	9.1	
Stn. 1		12–18	Boulders and rocks	13 500[f]	9.3	
Stn. 2		12–18	on sand	13 500[f]	7.2	
Narragansett Bay, Rhode Island	Summer	<10	Boulders	250	17.3	Fogarty 1976
		<10	Gravel	250	0	
		<10	Mussel bed on sand	250	0	
Long Island Sound	Year-round	7–9	Boulders on mud	5 500[g]	15.6	Stewart 1973

[a]Total area of all samples.
[b]Three sampling dates over 2 yr.
[c]Once each of 4 yr.
[d]Four dates.
[e]Six dates.
[f]25 successive months.
[g]24 consecutive months.

the importance of lobster predation on sea urchins, is summarized in Table 2. In all studies, data were collected by SCUBA divers surveying known areas of habitat. Because of the large and carefully measured survey areas and because size measurements were made on all lobsters collected, the best estimates were those of Scarratt (1968, 1973a, 1973b). Except for the Irish moss (*Chondrus crispus*) beds surveyed by Scarratt (1973a) and the gravel and mussel bed habitats surveyed by Fogarty (1976), all areas were chosen because they were known to have high lobster abundance or high yield to the fishery. In spite of the wide geographical range, the mean biomass measurements of the remainder of these sites were remarkably close to one another, clustered around $10\,g \cdot m^{-2}$. The Irish moss beds ($0.4–4.1\,g \cdot m^{-2}$) also supported commercial fishing but were not prime grounds (D. J. Scarratt, Biological Station, St. Andrews, N.B., pers. comm.).

Combining lobster predation rate on sea urchins with lobster biomass, we may infer that on good lobster grounds lobsters would kill 5–20 g sea urchins $\cdot m^{-2} \cdot yr^{-1}$. Using a production to biomass ratio of $0.8\,yr^{-1}$ for sea urchins (Miller and Mann 1973), the above predation would take the production from only $6–25\,g \cdot m^{-2}$, i.e. in a stable sea urchin population lobsters would account for the mortality from only this much of the population. However, these calculations best simulate the conditions on a sea urchin barren ground where the usual range of sea urchin biomass is a much greater $250–1100\,g \cdot m^{-2}$ (Chapman 1981; Bernstein and Welsford 1982) and sea urchins are exposed (Bernstein et al. 1981). This is because the only data available for calculation of lobster predation rates were based on high natural urchin density in the field and high density easily accessible in feeding experiments. No data have been published for lobster feeding rates on scarce or cryptic sea urchins, as sea urchins are found in seaweed beds (Bernstein et al. 1981; Wharton and Mann 1981), or for stomach contents of lobsters taken from seaweed beds. In the absence of these data we may conclude that there is meagre basis for a statement on lobster control of urchins in seaweed beds, or we may assume that lobster predation will be unimportant because prey availability is much lower than in the studies in Table 1 and lobster preference for urchins remains low. Contrary to the assumption of Miller et al. (1971), lobsters select sea urchins far below their relative abundance among prey species (Evans and Mann 1977; Elner 1980a; Carter and Steele 1982a).

Increasing the biomass of large lobsters, for example by

increasing the legal minimum size taken by the fishery or by reducing fishing intensity, would cause a much less than proportional increase in lobster predation on urchins. Sea urchins in the stomachs of lobsters 20–40 mm and >50 mm carapace length composed 0.2 and 0.05% of body weight, respectively (Scarratt 1980). Among lobster sizes of 60, 90, and 150 mm carapace length, weight-specific laboratory feeding rates on urchins were in the ratio of 8:3:1 (Elner 1980a). The minimum commercial size for lobsters in most of Nova Scotia is 81 mm carapace length. Furthermore, rock crabs, which are also sea urchin predators (see below), are important lobster prey (Ennis 1973; Breen 1974; Evans and Mann 1977; Scarratt 1980; Carter and Steele 1982a), and their prevalence in lobster stomachs greatly increases with lobster size (Scarratt 1980). At least qualitatively, sea urchins apparently have no refuge in size. Elner (1980a) fed a large range of urchin sizes (10–70 mm diameter) to three sizes each of lobsters and crabs. The larger lobsters and crabs included the largest urchins in their diets, and the smaller lobsters and crabs included the smallest urchins in their diets.

The simulation model presented by Breen (1974) and Breen and Mann (1976) will be given detailed consideration because important questions were identified and the results have often been quoted. As with most simulations of ecological systems, the data requirements were far greater than the investigators could reasonably measure. Some data and assumptions used may have biased high the decapod predation rates. In feeding experiments, crab, mussel, periwinkle, and starfish prey were stocked at $300\,g\cdot m^{-2}$, one to two orders of magnitude above measured field densities (Table 3 and Miller et al. 1971). The model simulation results were given for lobster densities set between 16 and $62\,g\cdot m^{-2}$, a range that may also be too high (Table 2). Lobsters were starved for 3–7 wk preceding 10 wk of feeding rate measurements. Finally, the stocking density of sea urchins as prey may have been high, as considered in the next section.

Predators and Sea Urchin Grazing Fronts

As constructed, the Breen and Mann (1976) model attached great importance to the urchin grazing front at the edge of the seaweed bed. The predator control of urchins was applied here and critical density for control was set at $2000\,g\cdot m^{-2}$. These choices were based on the observations that destructive grazing of a seaweed bed occurred at the edge and that $2000\,g\cdot m^{-2}$ was the minimum urchin density that caused the bed to recede. Because this critical density was based on observations on only one bed, within 1 yr, and only two data points were given for less than $2000\,g\cdot m^{-2}$, the result has a limited scope of inference notwithstanding an elegant method of measurement. However, given the estimate of critical density of $2000\,g\cdot m^{-2}$, the sea urchin stocking densities of $500–8000\,g\cdot m^{-2}$ used for lobster and crab feeding measurements ideally bracketed this value. The feeding rates of these predators increased with urchin density, and the model predicted that the lower the density at which the urchins were to be controlled the higher the lobster biomass required. Thus, if urchins were to be regulated at less than $2000\,g\cdot m^{-2}$, a greater lobster biomass would be required. The critical density for control may be much less than $2000\,g\cdot m^{-2}$.

The narrow strip of grazing front, usually only a few metres wide (Breen and Mann 1976; Bernstein et al. 1981), is a very small part of the sea urchin habitat. The lower urchin biomass and the population dynamics (recruitment, growth, and mortality) in adjoining areas of barren ground and seaweed bed might well be the critical parameters. This presumes that these areas supply the urchins to form the grazing front.

A good test of whether a barren ground has sufficient urchin density to graze a seaweed bed would be to maintain an urchin-free area within the barrens long enough for seaweed to become established, and then allow urchins from the barrens access to the new bed. Although I know of no data collected explicitly for this test, two studies reported relevant observations. In St. Margarets Bay, Chapman (1981) stocked two barren sites with 90 kg of kelp sori at a density of $15\,kg\cdot m^{-2}$, considerably higher than the usual natural density of about $3.5kg\cdot m^{-2}$ (Chapman 1981; Miller 1985a). Yet all the kelp was destroyed within 3 wk where surrounding urchin density was $1100\,g\cdot m^{-2}$ and within 11 wk where surrounding density was $250\,g\cdot m^{-2}$. In another barren area with an urchin density of $280\,g\cdot m^{-2}$, he maintained four 10-m^2 plots clear of urchins for 2.5 mo. The areas were grazed bare within 3 mo after removal stopped. In the St. Lawrence estuary, Himmelman et al. (1983a) kept urchins at far below ambient density for 2 yr. By the first observation 1 yr later, invading urchins had grazed the dominant *Alaria esculenta* from between the depths of 0.5 and 6 m (J. H. Himmelman, University of Laval, Quebec, Que., pers. comm.). This included *Alaria* densities to $3.3\,kg\cdot m^{-2}$. Urchin densities in the adjacent control area were $1200–2300\,g\cdot m^{-2}$.

Inside apparently persistent Nova Scotia kelp (*Laminaria* sp.) beds, urchin densities have been measured at approximately $80\,g\cdot m^{-2}$ (Wharton and Mann 1981), usually zero (Bernstein et al. 1981), and approximately $16\,g\cdot m^{-2}$ (Miller 1985a). Minimum densities of urchins that lead to destructive grazing within kelp beds have not been measured but have been estimated at 2–10 large individuals$\cdot m^{-2}$ (Wharton and Mann 1981) and 20–30 (probably large) individuals$\cdot m^{-2}$ (Bernstein et al. 1981). Even though we lack a good measure of critical urchin density either inside or outside a seaweed bed, and it may be similar, it probably lies in the hundreds rather than thousands of grams per square metre.

Mechanisms by which grazing fronts form have been given considerable attention, especially by Bernstein et al. (1981, 1983). These authors explained that when decapod predation pressure is reduced in seaweed beds, the density of sea urchins increases and they come out of hiding in search of food. Remaining decapod predators then cause these urchins to form defensive aggregations which graze holes in the bed, coalesce as grazing fronts, and if unchecked eliminate the bed. Fish predators can break up these aggregations.

The above studies have not supplied convincing evidence that predators are important in the formation or breakup of grazing aggregations. (Decapods are considered here and fish in a later section.) The formation of urchin aggregations within a seaweed bed has not been described. No comparative measure of decapod abundance in and out of seaweed beds or within seaweed beds over time was presented. Thus, no correlation of natural decapod abundance with urchin grazing intensity or with urchin aggregating behavior was (or is yet) available. Also, the effects of decapods on urchin grazing has not been studied in the laboratory.

The effect of decapods on urchin aggregating behavior in captivity has been studied. In laboratory tanks the percentage of urchins that were clumped (i.e. were touching at least one other urchin) increased in the presence of rock crabs (Bernstein et al. 1981). In cages in the field the percentage clumped sometimes increased in the presence of lobsters or rock crab and the mean

TABLE 3. Field biomass measurements in live weight for *Cancer irroratus*.

Location	Month	Depth (m)	Bottom type	Area sampled (m^2)[a]	Crab biomass ($g \cdot m^{-2}$)	Source
Southern Gulf of St. Lawrence	June and Sept.	4–18	Sand	1755	1.7	Scarratt and Lowe 1972
			Boulder	3762	3.6	
			Bedrock	3177	1.9	
Nova Scotia Atlantic coast	Aug.	1–9	Boulder	—	>60.0	Drummond-Davis 1978 Drummond-Davis et al. 1982
Nova Scotia Atlantic coast	Year-round	5–12	Boulder and cobble on sand and clay	260	3.9	Elner and Hamet 1984
Nova Scotia Atlantic coast	Sept.	4–8	Kelp bed, boulders on gravel	600	15.2	R. J. Miller, unpubl. data
		4–8	Barrens, boulders on gravel	600	12.3	
Narragansett Bay, Rhode Island	Summer	<10	Boulders	250	0	Fogarty 1976
		<10	Gravel	250	0	
		<10	Mussel bed on sand	250	17.2	

[a]Total area of all samples.

clump size sometimes increased in the presence of the same predators. The following data were taken from Bernstein et al. (1983, fig. 2, tables 5 and 6) and represent a cage population of 20 urchins:

	Predators		
Observation	Crabs	Lobsters	None
Percentage clumped in:			
kelp bed habitat	52	66	54
barren habitat	58	49	54
Mean clump size in:			
kelp bed habitat	1.8	2.3	1.8
barren habitat	2.2	1.7	1.8
Mean size of largest clump in:			
kelp bed habitat	5.2	7.4	4.7
barren habitat	6.3	3.6	4.8

If clumping of this degree does increase grazing, the small differences between treatments with and without predators do not suggest that predators would have an effect.

A simpler alternate hypothesis for the formation of urchin aggregations, one that does not require a behavioral response to predators, is as follows. The first bare patch in a seaweed bed could be formed by sea urchins settling in higher density in this patch, surviving in higher density in this patch, or eating the seaweed in this patch first because it is in lower density than in the surrounding area. Once the food supply within this patch was exhausted the urchins would gather at the newly formed edge of the seaweed and continue grazing. Thus, the grazing front is formed because that is where the food is. Many authors have reported how the green sea urchin quickly aggregates around and consumes isolated seaweed in both the field and laboratory (Himmelman and Steele 1971; Miller and Mann 1973; Vadas 1977; Himmelman 1980; Larson et al. 1980; Bernstein et al. 1981, 1983). None of these authors suggested that these aggregations were caused by predators.

The formation of new grazing fronts within seaweed beds is not necessarily a common phenomenon. Older fishermen reported that prior to recent mass mortalities, sea urchins were seldom rare on Nova Scotia lobster fishing grounds (Miller 1985a). In the first detailed survey of their abundance in 1968 (Mann 1972) they were widespread and locally abundant (Mann 1972; Miller and Mann 1973). Now that seaweed cover is widespread and urchin barrens are absent over large areas (Miller 1985a), there is a good opportunity to study the formation of these fronts.

The above hypothesis does not explain why nonfeeding aggregations form (Garnick 1978) or why feeding aggregations sometimes disperse (Bernstein et al. 1981). The above authors may be correct in their suggestions that these are responses to predators, although wave surge is another possibility. Himmelman and Steele (1971) reported that urchins that aggregated at the sublittoral fringe of algae were dispersed to deeper water by surge. Aggregations might also form in response to surge, since individuals within aggregations would offer less resistance to water movement than would isolated individuals. Tight aggregations in depressions are common in shallow, exposed locations (pers. obs.).

Rock Crab as Predator

The modest amount of data on rock crabs does not indicate that this is an important urchin predator. Of 171 crab stomachs with food taken from the southern Gulf of St. Lawrence, Scarratt and Lowe (1972) found urchin remains in 11. Drummond-Davis et al. (1982) examined 57 crab stomachs with food taken from a kelp bed near Halifax and found 2 with sea urchin remains. In the first study urchins were abundant in the habitat (D. J. Scarratt, pers. comm.); in the second they were not. When offered a choice of sea urchins and mussels in the laboratory, crabs killed about 0.4% (corrected for annual temperature range) of their body weight in urchins per day (Elner 1980a; Drummond-Davis et al. 1982), or equivalent to 1.5 times their body weight per year. However, when fed only sea urchins they killed a variable 1% (Himmelman and Steele 1971), 11%

TABLE 4. Stomach contents of fish caught in Coyle Cove in May, July, and August 1981 using trammel nets.

Species	Number of stomachs containing: food	sea urchin remains	sea urchin remains >10% of wet weight of total contents
Atlantic cod (*Gadus morhua*)	74	1	0
Atlantic tom cod (*Microgadus tomcod*)	7	0	0
Cunner (*Tautogolabrus adspersus*)	26	6	3
Little skate (*Raja erinacea*)	3	0	0
Pollock (*Pollachius virens*)[a]	24	0	0
Sculpins[b]	5	0	0
Silver hake (*Merluccius bilinearis*)	6	0	0
Winter flounder (*Pseudopleuronectes americanus*)	13	3	0
Total	158	10	3

[a]Fish >20 cm total length only; smaller fish are planktivorous.
[b]*Myoxocephalus octodecemspinosus*, *M. scorpius*, *Hemitripterus americanus*.

(Drummond-Davis et al. 1982) or 11, 16, or 36% (Elner 1980a) body weight per day, generally higher than the 1.2% (Himmelman and Steele 1971), 2–11% (Breen 1974), or 1.4–8% (Elner 1980a) body weight per day killed by lobsters fed only sea urchins.

In four studies, SCUBA divers measured crab biomass at 0–17.2 $g \cdot m^{-2}$ (Table 3). The results of Scarratt and Lowe (1972) and Elner and Hamet (1984) are probably the most typical of these. Fogarty's (1976) high value for a mussel bed represents a very small, very favorable portion of the habitat. The four experienced divers involved in the other survey (R. J. Miller, unpubl. data) all considered the crab density extraordinarily high. This area, in Louisbourg Harbour, may receive some organic subsidy from a fish meal processing plant about 1 km away. A fifth study (Drummond-Davis et al. 1982) estimated the biomass of large crabs at about 60 $g \cdot m^{-2}$ using baited traps and tag–recapture methods. However, Drummond-Davis (1978) expected that this value would have been much larger had the total range of crab sizes been included. The great differences between this study and the more direct diver survey results suggest some unresolved bias in the former.

If one accepts the laboratory predation rates where crabs were offered a choice of mussels and urchins, and the diver surveys of crab biomass, then rock crabs are probably responsible for killing <10 g urchins $\cdot m^{-2} \cdot yr^{-1}$. This low rate is supported by the two stomach analysis studies.

Fish as Predators

In this section an unreported study on fish stomach contents conducted in Coyle Cove, a small embayment near Halifax, will be discussed first, followed by a review of the literature on fish stomach contents, and finally a critique of a correlation of fish abundance and sea urchin behavior.

In the Coyle Cove study, fish were captured in trammel nets 25 m long by 1 m high set on rock bottom with sea urchins. Nets were fished perpendicular to shore for 2 h starting 1 h before sunset. Fish stomach contents were investigated from a total of 14 sets made in May, July, and August 1981. The minimum fish size vulnerable to capture was about 10 cm total length, but there was no upper limit.

Negligible amounts of sea urchin remains in fish stomachs from Coyle Cove (Table 4) indicate that fish predation was not important. Of 158 stomachs with food, only 10 had any sea urchin remains, and in only 3, all cunner (*Tautogolabrus adspersus*), did these exceed 10% of the weight of stomach contents. All species, except rock gunnel (*Pholis gunnellus*), commonly seen in the area during over 30 SCUBA dives were represented in the trammel net catches. The cryptic eel-like rock gunnels were occasionally collected in plankton nets towed along the bottom. Their stomachs contained principally amphipods.

A review of approximately 40 studies of gut contents of the species listed in Table 4 also indicates only small quantities of sea urchins eaten. In an eastern Newfoundland site, sea urchins composed 15% by weight of the gut contents of cunner (Green et al. 1984). In two Massachusetts studies, sea urchins were the fourth most important prey of cunner in frequency of occurrence (26–30%) (Chao 1973). At seven locations in eastern Canada, Johansen (1925) listed urchins in cunner guts as few or none. In two Canadian studies on winter flounder (*Pseudopleuronectes americanus*), sea urchins composed 1.5% by weight of gut contents (eastern Newfoundland) (Kennedy and Steele 1971) and negligible weight (western Bay of Fundy) (Wells et al. 1973). For the remaining fish species in Table 4 no reference was found that listed sea urchins as a significant prey item.

The Atlantic wolffish is a known predator of sea urchins (Bigelow and Schroeder 1953), but most data have been collected from offshore fishing banks (Bowman et al. 1976; Albikovskaya 1982). This species is a spring visitor to inshore waters in Nova Scotia but was seen in Coyle Cove on only 2 of over 30 dive days over 18 mo (pers. obs.). Thus, the species is a potentially important predator but only if its density and duration of stay in inshore waters is sufficient to have an impact.

Bernstein et al. (1981) proposed a cause–effect relationship from a correlation between a spring influx of predatory fish and dispersal of a sea urchin feeding front on the edge of a kelp bed in St. Margarets Bay; however, the correlation is questionable. Fish density was measured weekly for only 8 wk in the spring of 1980 and was high enough to correlate with other measures of sea urchin behavior for only 3–4 wk in May and early June. Sea urchin densities defining the presence of a feeding front were not provided for 1980. However, urchin

densities (number per square metre) were given for 1978 and 1979 (Bernstein et al. 1981, fig. 5) as follows:

1978	Density	1979	Density
January	18	April	22
May	45	June	8
June	40	July	27
August	90	November	20
December	100		

The marked features of these data are an increase through 1978, and then a precipitous decrease between December 1978 and April 1979, before the season when fish predators arrived in 1980. Another problem was identification of the predatory fish, reported as combined densities of wolffish and American plaice (*Hippoglossoides platessoides*). The flatfish was probably winter flounder because American plaice habitat is in temperatures of −1 to 5°C on soft bottom (Powles 1965), and winter flounder was the only flatfish caught in year-round sampling in a similar habitat in Coyle Cove. The winter flounder has a very small gape that would prevent it from preying on the large urchins in a feeding front.

Other Sea Urchin Predators

The green crab (*Carcinus maenas*) and Jonah crab (*Cancer borealis*) are moderately abundant in the western half of southern Nova Scotia (Elner 1980b; Elner and Hamet 1984; pers. obs.). The potential impact of Jonah crab was demonstrated in laboratory feeding trials at 8–12°C when they killed $8 \pm 3\%$ ($n = 2$ groups of 2 crabs $\times$ 19 d = 38) of their body weight per day when offered only urchins (L. D. Meade, Halifax Fisheries Research Laboratory, Halifax, N.S., pers. comm.). However, these data are not sufficient to estimate their predatory impact in nature. In general, crabs have greater dexterity than lobsters in preying on urchins (Himmelman and Steele 1971; Elner 1980a). Also, the relatively high density of small crabs with small chelae might make them effective hunters of small urchins in crevices.

Asterias sp. (Himmelman and Steele 1971; Bernstein et al. 1981), other green sea urchins, three species of sea gulls, and eiders (reviewed by Himmelman and Steele 1971) are all known predators of the green sea urchin in eastern Canada. Polychaetes, shrimp, and nemerteans can hunt cryptic habitats and might deserve consideration. The planktonic larvae no doubt have another suite of predators as suggested by Hooper (1980). More data are needed to judge the importance of some of these predators.

Summary

The information available provides a reasonably strong case that lobsters are unimportant predators of sea urchins, either now or in recent decades. For lobsters alone to have an impact on barren ground densities of sea urchins would require a lobster biomass several times higher than the $10\,g \cdot m^{-2}$ considered in this review to be high. Because of the ubiquity of the $10\,g \cdot m^{-2}$ maximum and relative unimportance of large lobsters as sea urchin predators, lobster populations previously exposed to lower fishing intensity probably did not control urchin populations either. Data are not available to calculate lobster predatory impact on low sea urchin densities living cryptically within seaweed beds. In the absence of these data we may leave the question open, or presume that lobster impact is still low because of continued low preference for urchins and low urchin availability.

Information on fish and crab predators could be expanded to more species and more locations, although available data do not rank these groups as important. A promising and neglected line of research for these and other urchin predators is predation success on small urchins which are given the opportunity to avoid predators in cryptic habitats.

Although predator-induced behavior remains a possible explanation for the formation and breakup of sea urchin grazing fronts, supporting data are weak and simpler explanations are consistent with the data.

Nonpredator Control of the Green Sea Urchin

Seaweeds always have small spatial refuges from urchin grazing (i.e. the urchins are controlled). These include shallow areas exposed to large waves, areas very sheltered from wave action, boulders among shifting sand, and low salinities. However, these refuges include ≤10% of the habitat in surveyed areas in southern Nova Scotia (Miller 1985a). Some algal species such as *Agarum cribrosum*, *Ptilota serrata*, and *Desmerestia viridis* are grazed less than others presumably because they are unpalatable (Vadas 1977; Himmelman 1980; Larson et al. 1980). However, they occupy only a small part of the habitat in Nova Scotia whether other species are abundant (Mann 1972) or scarce (Tremblay and Chapman 1980; Wharton and Mann 1981).

Seaweeds have recently had temporal refuge from grazing because of widespread urchin mass mortalities (Miller 1985a). From 1980 through 1983 nearly all sea urchins (approximately 245 000 t) died from disease within 0–15 m depth bordering 1400 km of shore. A significant but unmeasured fraction also died along another approximately 1000 km. In interviews, a few older fishermen recalled from the 1920's and 1930's that large numbers of urchin tests washed ashore, an occurrence likely associated with disease. Less drastic disease-induced mortalities might have occurred at other times but gone unnoticed. SCUBA diving became popular with marine scientists in Nova Scotia only in the mid-1960's.

Variable annual recruitment in the green sea urchin has been reported for Newfoundland (Himmelman 1969), Nova Scotia (Lang and Mann 1976), the St. Lawrence estuary (Himmelman et al. 1983b), and British Columbia (Foreman 1977). However, only in British Columbia was it suspected of being variable enough to affect urchin grazing.

In a general discussion of temperate fish predation on postlarval urchins, Choat (1982) concluded that disease and recruitment variability have greater effect on population structure than does predation.

Predator Control of Other Temperate Sea Urchins

In Lough Ine, Ireland, a suite of large decapod species were shown capable of eating the full range of sizes of *Paracentrotus lividus*. Judging from the distributions of predators and prey in the lough the decapods may have controlled urchin grazing in some parts (Muntz et al. 1965).

Recent studies in Alaska showed that by stampeding and eating *Strongylocentrotus* sp. the starfish *Pyncnopodia helianthoides* might reduce urchin grazing on localized areas enough to permit algal colonization (Duggins 1983). A predation rate

calculated from prey and predator density, handling time, and fraction of starfish feeding on urchins also implicated it as an important predator. Dayton (1975) earlier described *P. helianthoides* stampeding *Strongylocentrotus purpuratus* in tide pools and both the starfish and a sea anemone eating the sea urchins. The actions of these predators and the usual low mobility of the urchins combined to allow small patches of algal colonization.

The example of greatest predator impact is control of *Strongylocentrotus* sp. by the sea otter *Enhydra lutris*. Several authors (e.g. McLean 1962; Lowry and Pearce 1973; Estes et al. 1978; Breen et al. 1982) have reported for Alaska, British Columbia, and California numerous and strong negative correlations of sea otter and sea urchin abundances and have often observed otters eating urchins.

Predator control of urchins can permit continued maintenance of abundant seaweed or mediate change from barrens to seaweed cover. The decapod study was an example of the former whereas the starfish studies were the latter. The sea otter reports include examples of both.

Predation by spiny lobster (*Panulirus interruptus*) and/or a fish, the California sheephead (*Semicossyphus pulcher*), produced a bimodal size frequency distribution of *Strongylocentrotus franciscanus* in a southern California kelp bed (Tegner and Dayton 1981). However, data supporting claims that spiny lobster and sheephead regulate abundance of *S. franciscanus* and *S. purpuratus* sufficiently to control their grazing are equivocal. The authors inferred that predators controlled urchin grazing at two stations where predators were seen, but not at the third station where predators were not seen. However, the third station had the lowest density of both urchin species and the highest ratio of broken tests (evidence of sheephead and lobster predation) to live urchins (Tegner and Dayton 1981, table 2). Laboratory feeding rates of lobsters on *S. franciscanus* and *S. purpuratus* were quite high (Tegner and Levin 1983), but the absence of choice of prey, refuges for urchins, or field abundance of lobsters makes it difficult to apply these results to the field.

Large community investigations of fish stomach contents have shown sea urchins to be a minor component of fish diets. On Amchitka Island in the Aleutians, *Strongylocentrotus polycanthus* was found only incidentally in the stomachs of 25 species and was not among the 19 most important prey categories (Simenstad et al. 1977). For 19 species of benthic feeding fish from a British Columbia kelp bed, sea urchin prey were not mentioned (Leaman 1980). Of the 18 most abundant fish species sampled in southern Chile *Macrocystis* beds, none had urchins in their stomachs (Moreno and Jara 1984).

For a sea urchin-dominated barren ground in New Zealand, Andrew and Choat (1982) concluded that urchin predators did not play a key role in structuring the community. Fish were known to prey on juvenile urchins, and juvenile urchin density increased threefold in 14 mo when protected from fish by cages. However, because uncaged control sites did not develop algae and uncaged sites where urchins were removed developed lush algal growth, the authors concluded that enough juveniles survive to adulthood to maintain the habitat in a barren mode.

Of 45 fish species sampled from southern California kelp beds, sea urchins occurred in the guts of five species; but only in the California sheephead did they occur in more than 12% of the stomachs examined (Quast 1968). Three other California studies considered specifically sheephead predation on urchins. The study by Tegner and Dayton (1981) was discussed above. Nelson and Vance (1979) reasoned that the urchin *Centrostephanus coronatus* was limited to night foraging by sheephead predation. Cowen (1983) calculated that sheephead annually ate about 25% of *S. franciscanus* individuals in a kelp bed. He based the calculation on increased numbers in a predator removal area, predator feeding rates, and predator density.

Lobsters and Seaweed in Nova Scotia

In St. Margarets Bay, the only location in Nova Scotia where a time series of more than a few years is available, seaweed abundance is correlated with commercial landings of lobsters over the years 1968–83. Lobster landings for the east and west sides of the Bay are recorded in Statistical Districts 23 and 25, respectively. Figure 1 shows a decrease through the 1960's and 1970's and then a sharp upturn beginning in 1981. Because annual mortality rates (fishing plus natural) for lobsters are 40–86% (Campbell 1980), landings are expected to reflect abundance. To plot the relative abundance of seaweed (Fig. 1), the results of the first survey in 1968 (Mann 1972) were set equal to 100%. This abundance was reduced to 40% by 1973 (Breen and Mann 1976) and to 10% by 1978 (Chapman 1981). Although no more quantitative surveys were made, one would expect that seaweed remained at a low level until after sea urchin mass mortalities in 1980–81. In Coyle Cove, about 15 km to the east of St. Margarets Bay, seaweed biomass recovered from 15 to 30% of the biomass of a mature bed from 1981 to 1982 and to 50% by 1983 (Miller 1985a). Qualitative observations in St. Margarets Bay (Moore and Miller 1983; pers. obs.) indicate a similar recovery.

Breen and Mann (1976) and Wharton and Mann (1981) previously related lobster landings and seaweed abundance in St. Margarets Bay and proposed cause–effect relationships. As discussed earlier they argued that a pre-1968 decrease in lobster abundance caused by overfishing or environmental factors released urchins from predator control, permitting them to overgraze the seaweed. They also proposed that seaweed enhances lobster production. This proposal was defended with the reasonable arguments that seaweed adds third-dimensional complexity to the rock habitat, sheltering small lobsters from predators, and the large benthic plant production of a mature seaweed bed compared with barrens (Chapman 1981) increases the energy flow to lobsters. Also, many fishermen support the idea that kelp beds are the best place to catch lobsters. However, boulders and cobble may offer sufficient habitat complexity for small lobsters to escape predators, phytoplankton production might be adequate to fuel the food chain to lobsters, and lobsters' supposed preference for kelp beds may be a concentration at a prominent ecotone at the edge rather than an increase in biomass over a larger area. Fishermen I have interviewed on this subject stated a preference for setting traps at the edge of kelp beds.

Wharton and Mann (1981) also defended the following correlations: "From the records of American lobster (*H. americanus*) catches it was concluded that a critical decline in catches at each site occurred a few years after kelp bed destruction." In St. Margarets Bay, the only study site with a good time series of seaweed abundance, they dated destruction as most active from 1972 to 1975. However, the years of critical decline were given as 1968 and 1970 for Districts 25 and 23, respectively (Wharton and Mann 1981, fig. 4), both before their dates of active destruction.

If seaweed and lobster production have a cause–effect relationship the recent return of seaweed should, after some lag, increase lobster production. As seen in Fig. 1, lobster catches

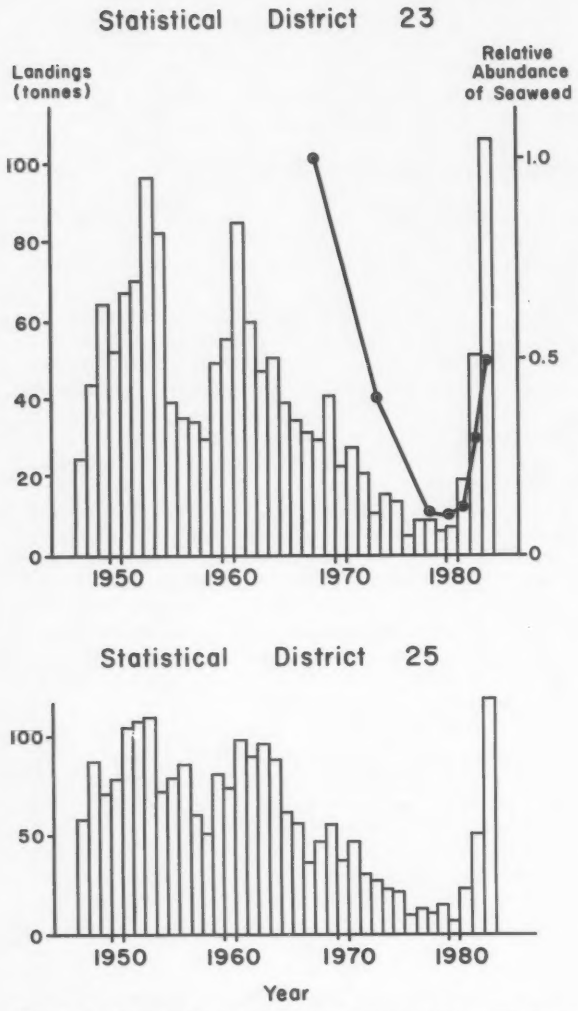


FIG. 1. Lobster landings for Statistical Districts 23 and 25 (bars) (landings of lobsters caught offshore during 1971–75 were removed from Ditrict 25 totals) and relative abundance of seaweed in St. Margarets Bay (solid line).

more than doubled from 1980 to 1981, but the seaweed recovery was modest until 1982. Enhancement of the lobster population through higher survival of young or higher habitat carrying capacity would not be seen in the landings until the mid- or late 1980's (lobsters of minimum commercial size are about 8 yr old) (Wilder 1953). The strong upturn in landings in 1981–83 were based on lobsters that were spawned and lived their early years in the mid-1970's when seaweed was at a low level and decreasing. In Statistical District 7, 300 km east of St. Margarets Bay, all but 1 of 12 sites surveyed in 1983 were classic sea urchin-dominated barrens (Miller 1985b; pers. obs.); however, lobster landings also began increasing there in 1981 and by 1983 were the highest since the statistical districts were formed in 1947.

Direct evidence that seaweed enhances lobster production is also lacking. Surprisingly, no comparative study of lobster abundance, feeding, food availability, growth, or survival in and out of seaweed beds has been published. A minor exception related to food availability is that more periwinkles were found in a seaweed bed than on an adjacent barrens (Bernstein et al. 1981). Periwinkles are only a minor item in lobster diet (Scarratt 1980; Carter and Steele 1982a). In the autumn of 1983 the abundance of juvenile and adult lobsters in a kelp bed and nearby barrens was estimated in Louisbourg Harbour, eastern Nova Scotia (Table 2). Fifteen transects totalling 600 m^2 were surveyed by divers in each area and provided similar mean biomass values of 12.8 $g \cdot m^{-2}$ in kelp and 9.8 $g \cdot m^{-2}$ in barrens ($t = 0.8$, $p > 0.05$).

In summary, seaweed may yet be shown to enhance the lobster population on Nova Scotia's south coast, but, to date, this relationship has only weak support from available data. More comparative surveys of lobster abundance in and out of seaweed beds should be carried out. These should include lobsters as small as practical. If possible, field experiments should be used to test the hypotheses that seaweed cover and primary production are beneficial to lobsters. A measure of more food or cover will not in itself demonstrate that more lobsters will use the habitat. Other factors expected to affect lobster abundance in southern Nova Scotia, especially recruitment overfishing and drift of larvae from the Gulf of St. Lawrence, have been recently reviewed by Campbell and Robinson (1983) and Harding et al. (1983).

Acknowledgements and Apologies

P. A. Breen, J. H. Choat, N. T. Hagan, and two anonymous reviewers provided helpful comments on the manuscript. J. H. Himmelman made unpublished material available. Researchers on the west coast of North America have conducted good studies on sea urchin aggregation and grazing. However, these have been capably reviewed by others and omitted from this review because of its focus on the green sea urchin.

References

ALBIKOVSKAYA, L. K. 1982. Characteristics of feeding of three species of wolffishes in the Northwest Atlantic. Northwest Atl. Fish. Org. SCR Doc. 82/VI/69: 9 p.

ANDREW, N. L., AND J. H. CHOAT. 1982. The influence of predation and cospecific adults on the abundance of juvenile *Evechinus chloroticus* (Echinoidea: Echinometridae). Oecologia 54: 80–87.

ANONYMOUS. 1887. Report of the commissioners, lobster and oyster fisheries of Canada. Can. House of Commons, Ottawa, Ont. 65 p.

BERNSTEIN, B. B., AND A. CAMPBELL. 1983. Contribution to the development of methodology for sampling and tagging small juvenile lobsters (*Homarus americanus*). Can. MS Rep. Fish. Aquat. Sci. 1741: 34 p.

BERNSTEIN, B. B., S. C. SCHROETER, AND K. H. MANN. 1983. Sea urchin (*Strongylocentrotus droebachiensis*) aggregating behaviour investigated by a subtidal multifactorial experiment. Can. J. Fish. Aquat. Sci. 40: 1975–1986.

BERNSTEIN, B. B., AND R. W. WELSFORD. 1982. An assessment of feasibility of using high-calcium quicklime as an experimental tool for research into kelp bed/sea urchin ecosystems in Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 968: 51 p.

BERNSTEIN, B. B., B. E. WILLIAMS, AND K. H. MANN. 1981. The role of behavioral responses to predators in modifying urchins' (*Strongylocentrotus droebachiensis*) destructive grazing and seasonal foraging patterns. Mar. Biol. 63: 39–49.

BIGELOW, H. B., AND W. C. SCHROEDER. 1953. Fishes of the Gulf of Maine. U.S. Dep. Inter. Fish Wildl. Serv. Fish. Bull. 74: 677 p.

BOWMAN, R. E., R. O. MAURER, JR., AND J. A. MURPHEY. 1976. Stomach contents of twenty-nine fish species from five regions in the northwest Atlantic — data report. Northeast Fishery Center, Woods Hole, MA., U.S. Natl. Mar. Fish. Serv. Data Rep. 76-10: 37 p.

BREEN, P. A. 1974. Relations among lobsters, sea urchins and kelp in Nova Scotia. Ph.D. thesis, Dalhousie University, Halifax, N.S. 198 p.

1980. Relations among lobster, sea urchins, and kelp in Nova Scotia,

p. 24–32. *In* J. D. Pringle, G. J. Sharp, and J. F. Caddy [ed.] Can. Tech. Rep. Fish. Aquat. Sci. 954: 273 p.

BREEN, P. A., T. A. CARSON, J. B. FOSTER, AND E. A. STEWART. 1982. Changes in subtidal community structure associated with British Columbia sea otter transplants. Mar. Ecol. Prog. Ser. 7: 13–20.

BREEN, P. A., AND K. H. MANN. 1976. Changing lobster abundance and destruction of kelp beds by sea urchins. Mar. Biol. 34: 137–142.

CARTER, J. A., AND D. H. STEELE. 1982a. Stomach contents of immature lobsters (*Homarus americanus*) from Placentia Bay, Newfoundland. Can. J. Zool. 60: 337–347.

1982b. Attraction to and selection of prey by immature lobsters (*Homarus americanus*). Can. J. Zool. 60: 326–336.

CAMPBELL, A. 1980. A review of mortality estimates of lobster populations in the Canadian Maritimes. Can. Tech. Rep. Fish. Aquat. Sci. 932: 27–38.

CAMPBELL, A., AND D. G. ROBINSON. 1983. Reproductive potential of three American lobster (*Homarus americanus*) stocks in the Canadian Maritimes. Can. J. Fish. Aquat. Sci. 40: 1958–1967.

CHAO, L. N. 1973. Digestive system and feeding habits of the cunner, *Tautogolabrus adspersus*, a stomachless fish. Fish. Bull. 71: 565–585.

CHAPMAN, A. R. O. 1981. Stability of sea urchin dominated barren grounds following destructive grazing of kelp in St. Margarets Bay, eastern Canada. Mar. Biol. 62: 307–311.

CHOAT, J. H. 1982. Fish feeding and the structure of benthic communities in temperate waters. Annu. Rev. Ecol. Syst. 13: 429–449.

COOPER, R. A., R. A. CLIFFORD, AND C. D. NEWELL. 1975. Seasonal abundance of the American lobster, *Homarus americanus*, in the Booth Bay region of Maine. Trans. Am. Fish. Soc. 104: 669–674.

COWEN, R. K. 1983. The effect of sheephead (*Semicossyphus pulcher*) predation on red sea urchins (*Strongylocentrotus franciscanus*) populations: an experimental analysis. Oecologia 58: 249–255.

DAYTON, P. K. 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. Ecol. Monogr. 45: 137–159.

DRUMMOND-DAVIS, N. 1978. Population estimates of the rock crab, *Cancer irroratus*, in a kelp bed and some aspects of its feeding. M.Sc. thesis, Dalhousie University, Halifax, N.S. 50 p.

DRUMMOND-DAVIS, N. C., K. H. MANN, AND R. A. POTTLE. 1982. Some estimates of population density and feeding habits of the rock crab, *Cancer irroratus*, in a kelp bed in Nova Scotia. Can. J. Fish. Aquat. Sci. 39: 636–639.

DUGGINS, D. O. 1983. Starfish predation and the creation of mosaic patterns in a kelp-dominated community. Ecology 64: 1610–1619.

ELNER, R. W. 1980a. Predation of the sea urchin (*Strongylocentrotus droebachiensis*) by the American lobster (*Homarus americanus*) and the rock crab (*Cancer irroratus*), p. 48–65. *In* J. D. Pringle, G. J. Sharp, and J. F. Caddy [ed.] Can. Tech. Rep. Fish. Aquat. Sci. 954: 273 p.

1980b. The diet of the green crab *Carcinus maenas* (L.), from Port Hebert, southwestern Nova Scotia. J. Shellfish Res. 1: 89–94.

ELNER, R. W., AND S. L. HAMET. 1984. The effects of ocean dumping of dredge spoils onto juvenile lobster habitat: a field evaluation. Can. Tech. Rep. Fish. Aquat. Sci. 1247: 12 p.

ENNIS, G. P. 1973. Food, feeding, and condition of lobsters, *Homarus americanus*, throughout the seasonal cycle in Bonavista Bay, Newfoundland. J. Fish. Res. Board Can. 30: 1905–1909.

ESTES, J. A., N. S. SMITH, AND J. F. PALMISANO. 1978. Sea otter predation and community organization in the western Aleutian Islands, Alaska. Ecology 59: 822–833.

EVANS, P. D., AND K. H. MANN. 1977. Selection of prey by American lobsters (*Homarus americanus*) when offered a choice between sea urchins and crabs. J. Fish. Res. Board Can. 34: 2203–2207.

FOGARTY, M. J. 1976. Competition and resource partitioning in two species of *Cancer* (Crustacea, Brachiyura). M.Sc. thesis, University of Rhode Island, Kingston, RI. 96 p.

FOREMAN, R. E. 1977. Benthic community modification and recovery following intensive grazing by *Strongylocentrotus droebachiensis*. Helgol. Wiss. Meeresunters. 30: 468–484.

GARNICK, E. 1978. Behavioral ecology of *Strongylocentrotus droebachiensis* (Muller) Echinodermata: Echinoidea. Oecologia 37: 77–84.

GREEN, J. M., G. MARTEL, AND D. W. MARTIN. 1984. Comparisons of the feeding activity and diets of male and female cunners *Tautogolabrus adspersus* (Pices: Labridae). Mar. Biol. 84: 7–11.

HARDING, G. C., K. F. DRINKWATER, AND W. P. VASS. 1983. Factors influencing the size of American lobster (*Homarus americanus*) stocks along the Atlantic coast of Nova Scotia, Gulf of St. Lawrence, and Gulf of Maine: a new synthesis. Can. J. Fish. Aquat. Sci. 40: 168–184.

HIMMELMAN, J. H. 1969. Some aspects of the ecology of *Strongylocentrotus droebachiensis* in eastern Newfoundland. M.Sc. thesis, Memorial University of Newfoundland, St. John's, Nfld. 159 p.

1980. The role of the green sea urchin, *Strongylocentrotus droebachiensis*, in the rocky subtidal region of Newfoundland, p. 92–119. *In* J. D. Pringle, G. J. Sharp, and J. F. Caddy [ed.] Can. Tech. Rep. Fish. Aquat. Sci. 954: 273 p.

HIMMELMAN, J. H., A. CARDINAL, AND E. BOURGET. 1983a. Community development following removal of urchins, *Strongylocentrotus droebachiensis*, from the rocky subtidal zone of the St. Lawrence estuary, eastern Canada. Oecologia 59: 27–39.

HIMMELMAN, J. H., Y. LAVERGNE, F. AXELSEN, A. CARDINAL, AND E. BOURQUET. 1983b. Sea urchins in the St. Lawrence estuary: their abundance, size structure, and suitability for commercial exploitation. Can. J. Fish. Aquat. Sci. 40: 474–486.

HIMMELMAN, J. H., AND D. H. STEELE. 1971. Foods and predators of the green sea urchin (*Strongylocentrotus droebachiensis*) in Newfoundland waters. Mar. Biol. 9: 315–322.

HOOPER, R. 1980. Observations on algal–grazer interactions in Newfoundland and Labrador, p. 120–124. *In* J. D. Pringle, G. J. Sharp, and J. F. Caddy [ed.] Can. Tech. Rep. Fish. Aquat. Sci. 954: 273 p.

JOHANSEN, F. 1925. Natural history of the cunner (*Tautogolabrus adspersus* Walbaum). Contrib. Can. Biol. 2: 427–468.

JOHNSON, C. R., AND K. H. MANN. 1982. Adaptation of *Strongylocentrotus droebachiensis* for survival on barren grounds in Nova Scotia, p. 277–283. *In* J. M. Lawrence [ed.] Int. Echinoderm Conf.

KENNEDY, V. S., AND D. H. STEELE. 1971. The winter flounder, *Pseudopleuronectes americanus*, in Long Pond, Conception Bay, Newfoundland. J. Fish. Res. Board Can. 28: 1165–1183.

LANG, C., AND K. H. MANN. 1976. Changes in sea urchin populations after the destruction of kelp beds. Mar. Biol. 36: 321–326.

LARSON, B. R., R. L. VADAS, AND M. KESER. 1980. Feeding and nutritional ecology of the sea urchin *Strongylocentrotus droebachiensis* in Maine, U.S.A. Mar. Biol. 59: 49–62.

LEAMAN, B. M. 1980. The ecology of the fishes in British Columbia kelp beds. I. Barkley Sound *Nereocystis* beds. Fish. Dev. Rep. 22, Marine Res. Branch, Min. Environ., Prov. of British Columbia, Victoria, B.C.

LOWRY, L. F., AND J. S. PEARCE. 1973. Abalones and sea urchins in an area inhabited by sea otters. Mar. Biol. 23: 213–219.

MANN, K. H. 1972. Ecological energetics of the seaward zone in a marine bay on the Atlantic coast of Canada. I. Zonation and biomass of seaweed. Mar. Biol. 12: 1–10.

1977. Destruction of kelp beds by sea urchins: a cyclical phenomenon or irreversible degradation? Helgol. Wiss. Meeresunters. 30: 355–467.

1982. Kelp, sea urchins, and predators: a review of strong interactions in rocky subtidal systems of eastern Canada, 1970–1980. Neth. J. Sea Res. 16: 414–423.

MANN, K. H., AND P. A. BREEN. 1972. The relationship between lobster abundance, sea urchins, and kelp beds. J. Fish. Res. Board Can. 29: 603–609.

MCLEAN, J. H. 1962. Sublittoral ecology of the open coast near Carmel, California. Biol. Bull. 122: 95–114.

MCLEESE, D. W., AND D. G. WILDER. 1958. The activity and catchability of the lobster (*Homarus americanus*) in relation to temperature. J. Fish. Res. Board Can. 15: 1345–1354.

MILLER, R. J. 1985a. Succession of sea urchin and seaweed abundance in Nova Scotia, Canada. Mar. Biol. 84: 275–286.

1985b. Sea urchin pathogen: a possible tool for biological control. Mar. Ecol. Prog. Ser. 21: 169–174.

MILLER, R. J., AND K. H. MANN. 1973. Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. III. Energy transformations by sea urchins. Mar. Biol. 18: 99–114.

MILLER, R. J., K. H. MANN, AND D. J. SCARRATT. 1971. Production potential of a seaweed–lobster community in eastern Canada. J. Fish. Res. Board Can. 28: 1733–1738.

MOORE, D. S., AND R. J. MILLER. 1983. Recovery of macroalgae following widespread sea urchin mortality with a description of the shallow, hard-bottom habitat on the Atlantic coast of Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 1230: 94 p.

MORENO, C. A., AND H. F. JARA. 1984. Ecological studies on fish fauna associated with *Macrocystis pyrifera* belts in the south of Fuequian Islands, Chile. Mar. Ecol. Prog. Ser. 15: 99–107.

MUNTZ, L., F. J. EBLING, AND J. A. KITCHING. 1965. The ecology of Lough Ine. XIV. Predatory activity of large crabs. J. Anim. Ecol. 34: 315–329.

NELSON, B. V., AND R. R. VANCE. 1979. Diel foraging patterns of the sea urchin *Centrostephanus coronatus* as a predator avoidance strategy. Mar. Biol. 51: 251–258.

POWLES, P. M. 1965. Life history and ecology of American plaice (*Hippoglossoides platessoides* F.) in the Magdalen Shallows. J. Fish. Res. Board Can. 22: 565–598.

PRINGLE, J. D., G. J. SHARP, AND J. F. CADDY. 1980. An update on the relationship between commercial lobstering and urchin and macroalgal densities, p. 237–251. *In* J. D. Pringle, G. J. Sharp, and J. F. Caddy [ed.] Can. Tech. Rep. Fish. Aquat. Sci. 954: 273 p.

QUAST, J. C. 1968. Observations on the food of the kelp bed fishes. Calif. Dep. Fish Game Fish Bull. 139: 57–79.

SCARRATT, D. J. 1968. An artificial reef for lobsters (*Homarus americanus*). J. Fish. Res. Board Can. 25: 2683–2690.

1973a. The effects of raking Irish moss (*Chondrus crispus*) on lobsters in Prince Edward Island. Helgol. Wiss. Meeresunters. 24: 415–424.

1973b. Lobster populations on a man made rocky reef. Int. Coun. Explor. Sea Res. Doc. C.M. 1973/K:47: 3 p.

1980. The food of lobsters, p. 69–91. *In* J. D. Pringle, G. J. Sharp, and J. F. Caddy [ed.] Can. Tech. Rep. Fish. Aquat. Sci. 954: 273 p.

SCARRATT, D. J., AND R. LOWE. 1972. Biology of rock crab (*Cancer irroratus*) in Northumberland Strait. J. Fish. Res. Board Can. 29: 161–166.

SIMENSTAD, C. A., J. S. ISAKSON, AND R. A. NAKATANI. 1977. Marine fish communities of Amchitka Island, Alaska, p. 451–492. *In* M. L. Merritt and R. G. Fuller [ed.] The environment of Amchitka Island. TID-26712, Natl. Tech. Infor. Serv., U.S. Dep. Commerce, Springfield, VA.

STEWART, L. L. 1973. The seasonal movements, population dynamics and ecology of the lobster, *Homarus americanus* (Milne-Edward), off Ram Island, Connecticut. Ph.D. dissertation, University of Connecticut, Storrs, CT. 112 p.

TEGNER, M. J., AND P. K. DAYTON. 1981. Population structure, recruitment and mortality of two sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*) in a kelp forest. Mar. Ecol. Prog. Ser. 5: 255–268.

TEGNER, M. J., AND L. A. LEVIN. 1983. Spiny lobsters and sea urchins: analysis of a predator–prey interaction. J. Exp. Mar. Biol. Ecol. 73: 125–150.

THOMAS, J. C. 1973. Analysis of the commercial lobster (*Homarus americanus*) fishery along the coast of Maine, August 1966 through December 1970. NOAA Tech. Rep. NMFS SSRF 667: 57 p.

THOMPSON, R. J. 1983. The relationship between food ration and reproductive effort in the green sea urchin, *Strongylocentrotus droebachiensis*. Oecologia 56: 50–57.

TREMBLAY, C., AND A. R. O. CHAPMAN. 1980. The local occurrence of *Agarum cribrosum* in relation to the presence or absence of its competitors or predators. Proc. N.S. Inst. Sci. 30: 165–170.

TRITES, R. W. 1982. Overview of oceanographic conditions of NAFO Subarea 2, 3, and 4 during the 1970–1979 decade. Northwest Atl. Fish. Org. SCS Doc. 5: 51–78.

VADAS, R. L. 1977. Preferential feeding: an optimization strategy in sea urchins. Ecol. Monogr. 47: 337–371.

WELLS, B., D. H. STEELE, AND A. V. TYLER. 1973. Intertidal feeding of winter flounders (*Pseudopleuronectes americanus*) in the Bay of Fundy. J. Fish. Res. Board Can. 30: 1374–1378.

WHARTON, W. G., AND K. H. MANN. 1981. Relationship between destructive grazing by the sea urchin, *Strongylocentrotus droebachiensis*, and the abundance of American lobster, *Homarus americanus*, on the Atlantic coast of Nova Scotia. Can. J. Fish. Aquat. Sci. 38: 1339–1349.

WILDER, D. G. 1953. The growth rate of the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 10: 371–412.

BOOK REVIEWS/COMPTES RENDUS

QSAR in Environmental Toxicology. *Klaus L. E. Kaiser (editor). D. Reidel Publishing Company, Dordrecht, Boston, Lancaster. 1984. xiv + 406 p. 145 (DFI), $54.50 (U.S.).*

This volume constitutes the published proceedings of a workshop on quantitative structure – activity relationships (QSAR) in environmental toxicology held August 16–18, 1983, at McMaster University, Hamilton, Ont., Canada, and attended by aquatic biologists and toxicologists from Canada, the United States, and Europe. The purpose of the meeting was to relate the known physical and chemical properties of existing environmental contaminants found in stream, river, and lake water to the toxicity observed in aquatic life, using various simple and complex environmental models associated with mathematical treatment or actual experimentation.

Whereas much of the data has been published before, this volume contains probably everything that you ever wanted to know about the subject gathered together between two covers. It is particularly useful for the uninitiated scientist looking for an overview of the topic or knowledge of the state of the art. The major concerns in the volume center around the multitude of chlorinated aromatic (benzenes, biphenyls, dioxins, dibenzofurans, phenols, etc.) and aliphatic (alkanes, alkenes, etc.) contaminants, these receiving considerable attention in the various chapters. The diversity of chemicals defies the establishment of reasonable patterns of QSAR once one goes beyond homologous series of chemicals. Some venturesome scientists addressed themselves to broader fields, with the development of variations of the Hansch model that work reasonably well for many of the chemicals selected. Much of what was presented bears a strong resemblance to the SAR studies conducted years ago on drug molecules.

Simple and complex modelling has involved an interrelationship between four categories of properties and their linking equations, namely molecular descriptors, fundamental thermodynamic functions, physical–chemical properties, and environmental partitioning quantities. The type of data required for the prediction of the environmental fate of toxic contaminants in large lakes by mathematical models was discussed. At the other end of the spectrum, one group of authors described a system of simple, fast, and inexpensive laboratory tests designed to examine mammalian metabolism, bioaccumulation, fate in sewage, waste composting, and atmospheric breakdown. Using these tests, an environmental hazard profile could be developed for the ranking of any chemical with respect to the accumulation and degradation potential for any test species or environmental situation.

Some papers dealt with the toxicity of homologous series of chemicals in yeasts, bacteria, aquatic organisms including minnows and rainbow trout, and in rats. The results give the appearance of an "order to things in the world" but, as one should appreciate when working with homologues, slight changes in structure are reflected frequently in slight changes in physicochemical properties and in the subsequent distribution in the environment. In contrast, biotransformation plays a major role in interhomologue differences in toxicity, a point well demonstrated in the rat studies using chlorobenzene isomers.

This is not an easy book for the novice to read and it is best digested in small doses. The reviewer has always found it difficult to grasp concepts from overwhelming numbers in tabulated form, the hypothesis tested being much easier to visualize in a well-designed figure. There are few such figures in the book but a plethora of tables which detract somewhat from the appearance. However, the volume contains a vast and useful array of information concerning the physical and chemical properties of organic environmental contaminants which can be retrieved easily by consulting the excellent subject index.

As in any workshop, one of the important features is the open discussion betwen participants following the presentation of a paper. This difficult task was handled in superb fashion and the editor is to be congratulated on the clean presentation of the informative off-the-cuff remarks, a useful addition to the book.

D. J. ECOBICHON

McGill University
Pharmacology and Therapeutics
McIntire Medical Sciences Building
3655 Drummond Street
Montreal, Que. H3G 1Y6

(R622)

Proceedings of the National Aquaculture Conference. Strategies for aquaculture development in Canada. *G. I. Pritchard (editor). Can. Spec. Publ. Fish. Aquat. Sci. 75. 1984. 131 p. $8.00 (Canada).*

The National Aquaculture Conference, sponsored jointly by the Department of Fisheries and Oceans and the Science Council of Canada, was the largest and most comprehensive Canadian aquaculture conference to date. It was designed to provide an overview of existing aquaculture practices, to gather opinion on the function of aquaculture (socioeconomic force and/or profit-centered activity?), to provide direction for aquaculture development, and to initiate strategic planning.

The papers comprising this publication are organized, as was the conference, into themes covering the following: overviews of the diversity of regional aquaculture practices and their status; risk reduction; planning for aquaculture development; marketing; and socioeconomic dimensions — preceded by a keynote address, "Getting Our Act Together." The papers express a variety of viewpoints which ranged from mundane to provocative. The final paper, "Aquaculture — A Monetary Black Hole?" by Saxby, is an extraordinary mélange of opinion. If a reader wishes to garner some of the flavour of the conference or has time to read just one paper, this is it.

Saxby's paper, in its discussion of "Aquaculture Macroeconomics," describes possibilities for aquaculture which vary considerably, each possibility requiring a different level of government intervention. For example, if aquaculture is profitable (*Pisces pecuniaris*, the "money fish" referred to in the opening address), keep government hands off. If it is less profitable (for example due to market failures), government intervention may be warranted. In the most extreme case, government may see fit to use aquaculture to increase the productivity of welfare recipients (*Salmo socioeconomicus*?) (perhaps by distributing oyster rakes, rather than shovels, as has been suggested by one B.C. Cabinet Minister?). The point is that Saxby does not know which scenario is most likely, and seemingly, neither does anyone else. Everyone agrees, however, that aquaculture can or should contribute to the well-being of Canadians in one form or another.

Regional papers present a lot of data on the production of various species. However, the data appear to have been obtained from many different, often unspecified sources, are questionable in some cases, and apply to several divergent definitions of aquaculture. No comparison between these figures and those presented by MacCrimmon et al. (1974) was attempted. A summary of the production figures for all provinces and species would have been helpful to the reader. Nevertheless, one can discern the status of aquaculture on a regional basis from the discussions and speculation evident in each paper. No effort was made to attempt to project the potential production of any species in the near or distant future.

Since the conference, there has been an emphasis in the popular press on the science and technology (largely undefined) which may provide opportunities for aquaculture development. In addition, Science Council reports and media hyperbole have fostered the notion that simply developing a national policy for aquaculture and providing investment underwritten by government will result in numerous, viable aquaculture projects and the creation of thousands of new jobs. The conference proceedings provide a valuable counterbalance. Several authors point out that the present industry in Canada is based on simple technologies and a lot of hard work, that it is first and foremost farming, and will only develop slowly given favourable conditions and markets. The Ontario trout farming industry is perhaps the best example of this, having grown over the past decade from a production of 50 to an estimated 540 tonnes of rainbow trout or approximately one fifth of total Canadian aquaculture production. This development has not resulted from large, high-tech investments but rather from a number of small operations which have expanded as markets developed.

There are several areas in which the role of governments has been important in aquaculture and could be more so in the future. A number of contributors emphasize the need for government to fund promising research and development projects, to provide technology transfer from experience gained in its own operations, and in particular to support the essential pilot production phase for new approaches or species. Governments must also ensure that unnecessary constraints on the industry are removed and that some concerted planning for aquaculture development is carried out to provide clear policy and direction for proponents. Such planning must, however, be based on such obvious considerations as the real potential for industry growth including available markets, present or future, water supply and water temperature limitations, the supply of feed ingredients, and, of course, the available human resources.

Many recommendations to government, and some to the industry, were prepared at the conference and published separately (Anonymous 1983). It would have been useful to have included three pages from that document titled "Future Strategies" [for Aquaculture Development in Canada] with these proceedings, because without them, the reader is left with some confusion as to what the direction was indicated from the conference.

These proceedings are an important document for anyone interested in Canadain aquaculture. The reports of the failure of large projects and the high risk nature of the field do not inspire confidence for investors but the realistic identification of constraints and opportunities in specific areas will serve to guide the serious investor. Governments are affording commercial aquaculture a higher priority than it has received in the past, and steady but moderate growth can be expected with a number of species in suitable locations.

References

ANONYMOUS. 1983. Report of the Proceedings of the National Aquaculture Conference, St. Andrews, New Brunswick, July 10–14, 1983. 57 p.

MACCRIMMON, H. R., J. E. STEWART, AND J. R. BRETT. 1974. Aquaculture in Canada — the practice and the promise. Bull. Fish. Res. Board Can. 188: 84 p.

JULIAN HYNES AND ALLAN CASTLEDINE

Ontario Ministry of Natural Resources
Toronto, Ont. M7A 1W3 (R644)

Storm Surges — Meteorological Ocean Tides. *T. S. Murty. Can. Bull. Fish. Aquat. Sci. 212. 1984. 897 p. $34.95 (Canada), $41.95 (other countries).*

Popularly referred to as tidal waves or tidal bores in the media, storm surges, as they are known in the scientific literature, are responsible for the greatest loss of life on a world-wide basis of all natural disasters according to T. S. Murty. *Storm surges — Meteorological ocean tides* is intended to survey the scientific literature appearing in English and highlights the main findings of a vast number of investigations of storm surges and related topics such as the nature of the causative meterological systems. The author does not stop short of the technical side of this topic when he offers the reader his advice on the distribution of aid to victims of storm surges; a higher national authority is less prone to corruption than are local officials.

The reader may find the first part of this book, which introduces the underlying theoretical considerations and concepts of storm surges, more difficult to read on the whole than the second half, which discusses an abundance of case histories of individual storms and their consequent water level responses in various parts of the world including inland seas and lakes where storm surge incidence is notable. The book abounds throughout with bold and clearly labelled figures adapted from the research literature and should serve as a model of clarity for speakers at scientific meetings. In all cases, the units in the figures have been converted to the metric system. Whereas this book is directed mainly to the nonspecialist, each chapter should offer even the most seasoned practitioner some new material to ponder. For example, one of the themes of the book is the comparison of observed water level fluctuations during a storm period with those simulated mathematically either by statistical, numerical, or analytical means from observed or forecast meteorological input. It came as a surprise to me that one of the most successful predictions of coastal storm surges may be

made from knowledge of the tilts of the land surface inland from the coast. Other gems that may interest the specialist are the elegant mathematical theory of cyclone tracks (method of images) in the Bay of Bengal leading to "rose petal" storm trajectories and the meteorological phenomenon referred to in the book as "gusts bumps."

I strongly disagree with the author's preface that a subject index is not necessary since a table of contents provides sufficient detail. My experience was that the table of contents was far too brief as a guide to the subject material. Furthermore, due to the organization of the book a subject is often referred to in a number of chapters. For example, the reader who may wish to use the book to gather background information on some particular event such as the recent tragedy in Bangladesh (*MacLean's*, June 10, 1985) would find to his frustration that storm surges of that region are covered in Chapters 1, 3, 6, and 7 with no mention at all in the table of contents. A proper subject index would add immensely to the value of this book. A further shortcoming is that the over 1500 references comprising the extensive bibliography, almost all of which are cited in the text, are not linked to the source of citation. The occasional typographical error is an annoyance but not as serious as it would be in a monograph, since the reader can always refer to the original material.

A surprising omission was the detailed treatment of the oceanic storm surge prediction model, SPLASH, and its derivatives developed by the U.S. National Oceanic and Space Administration in consideration of the world-wide application that this method has received in providing surge warnings on an operational basis. Since only about 10% of the references date more recently than 1980, perhaps interest in research in this area is falling off. One could suppose that one of the author's motives was to spark renewed research interest in this apparently well-established field. It is, therefore, surprising that he does not offer us his thoughts on priority areas for future research on storm surges. Unfortunately, this matter is left to the reader to formulate.

Since the book is marred by the lack of a subject index, in my view the student of atmospheric sciences, oceanography, or ocean engineering who wished to gain easy access to the state of the art on storm surges would benefit most from it. Thus, it is recommended that this book be made available in university research libraries.

Even though the preparation of this extensive review would be greatly facilitated by computerized searches of science and engineering abstracts, the author is to be commended for his time and effort spent in his writing of this fine summary of the storm surge literature.

P. F. HAMBLIN

Physical Limnology Section
National Water Research Institute
Canada Centre for Inland Waters
867 Lakeshore Road
Burlington, Ont. L7R 4A6

(R620)

Communication and behavior of whales. *Edited by Roger Payne. Westview Press, Boulder, CO, for the American Association for the Advancement of Science. AAAS Selected Symposium 76. 1983. 643 p. $35.00 (U.S).*

This interesting and useful book is really about southern right whales (*Eubalaena australis*) and humpback whales (*Megaptera novaeangliae*) (12 of 14 chapters), with one chapter on grey whales (*Eschrichtius robustus*) and one on killer whales (*Orcinus orca*). Unlike many recent whale books, this one lives up to its title. It contains strong contributions in each of five sections: Vocalizations, Behavior on Breeding Grounds, Migratory Destinations and Stock Identification, Research Techniques, and a Bibliography.

Roger Payne is much more than an editor to this volume. He co-authored four of the 14 papers, and all but two cover work done either in association with his Center for Long Term Research in Lincoln, Massachusetts, or with a graduate-student project he initiated and oversaw in Hawaii. Payne has been the driving force behind what can legitimately be regarded as a movement within the politically and emotionally charged world of cetacean management and research. He believes that whales should, whenever possible, be studied alive and unrestrained. Thus, he is critical of the traditional approaches to studying whales, which usually involve the examination of carcasses ("corpses" as he calls them). The study of live whales promises to provide, in Payne's words, "important new insights, not just into the remains of the whale but into the rest of the whale, the part that has escaped the whalers for so long." Brash and unsettling as some of his claims may seem to old-guard cetologists, there is truth in them. This book is proof.

Part 1 on vocalizations contains three papers on humpbacks, one on killer whales, and one on right whales. The chapter by K. Payne, Tyack, and R. Payne hinges on a detailed analysis of the songs recorded from humpbacks in Hawaii during two successive winters. Although all whales on a particular wintering ground sing essentially the same song, elements of that song may change rapidly and synchronously over the course of a singing season. The authors conclude that the changes are "primarily cultural" rather than linked to natural rhythms or periodic phenomena in the whales' environment. The chapter by Guinee, Chu, and Dorsey documents the progressive changes made in the songs of three individually identified humpbacks, recorded at intervals of less than 1 yr. These individuals updated their songs so as to reflect the changes made by the rest of the group. Thus, song changes probably cannot be explained by year-to-year turnover in the population using a particular ground.

Clark's highly innovative work on the sounds of right whales is an important step toward understanding the functional significance of baleen whale vocalization. In a previous paper (1982, Anim. Behav. 30: 1060–1071), Clark summarized the acoustic repertoire of *Eubalaena australis*, but here he attempts to place the various sounds in their social context. He concludes that many of the right whale's sounds are used to communicate a complex array of messages. Intriguing questions are raised. For example, is there some variable in the contact call that encodes for the identity of the caller?

Part 2 includes two chapters on humpback behavior at the Hawaiian breeding grounds, one on grey whale behavior at lagoon entrances, and one on right whale behavior. The solid case study of humpback mothers and calves in Hawaii by Glockner and Venus is one of the first attempts to investigate the lives of whales through underwater observations and photography. It has provided unequivocal evidence of a 1-yr interbirth interval for some individuals of this species (1984, Rep. Int. Whaling Comm. Spec. Iss. 6: 237–242). Whether or not a 1-yr cycle is part of a density-dependent response in much-depleted populations, or is to be found as part of normal variation in

populations at equilibrium, is a research question of great importance to whale biology and management. Glockner's other chapter (in Part 4) demonstrates a method for determining the sex of adult humpbacks by the presence or absence of a protuberance posterior to the genital slit. This "hemispherical lobe" (or more appropriately, True's "hemispherical boss" (1904, Smithson. Contrib. Knowl. 33: 230)) can readily be seen in a lateral view of the abdomen, allowing researchers to sex humpbacks with good underwater photographs showing the whale in profile.

"Sexual Dimorphism and Aggressive Use of Callosities in Right Whales (*Eubalaena australis*)" by R. Payne and Dorsey is innovative in conception and carefully documented. The authors have developed a plausible explanation of one function of the right whale's callosities. If they are correct in interpreting the differences between the two sexes in size and extent of callosities, incidence of scrape marks, and behavior within "active mating groups," then the right whale is something more than a placid zooplankton grazer. Until recently, the conventional wisdom was that baleen whales were incapable of aggressive and injurious interactions. Prolonged observations of humpbacks on their winter grounds have now shown that males can be aggressive, at least during the season of reproduction (1983, Behaviour 83: 132–154; 1984, Can. J. Zool. 62: 1922–1937). With Payne and Dorsey's analysis, the right whale, too, must be considered in a new light. Male–male competition is a feature in the mating systems of two of the three mysticete species, each representing a taxonomically different family, whose breeding behavior has now been closely observed (the grey whale being the third).

The two chapters in Part 3 on migratory destinations and stock identity focus on humpbacks in the central and eastern North Pacific. Darling and Jurasz use fluke photographs, now a standard tool for assessing humpback movements, to show that some humpbacks which summer in the Gulf of Alaska winter in Hawaii and near the Revillagigedo Islands off Mexico (also see 1985, Can. J. Zool. 63: 308–314). One whale was found wintering in Hawaii one year and at the Revillagigedos 2 yr later. This is taken to mean that the whales using these two wintering grounds 4800 km apart belong to one stock.

"Humpback Whale (*Megaptera novaeangliae*) Songs as an Indicator of 'Stocks'" by R. Payne and Guinee uses a less straightforward, and in our view less conclusive, approach to proving the same thing. Similarities in songs recorded in Hawaii and at the Revillagigedos are regarded as evidence that whales from the central and eastern North Pacific belong to one stock. Payne and Guinee discuss various ways in which the whales from two distant wintering areas might come into close enough physical proximity to develop a similar song each year. They lay to rest the possibility that humpback songs might be heard across several thousand kilometres of distance, as has been suggested by Payne and Webb (1971, Ann. N.Y. Acad. Sci. 188: 110–142) for the low-frequency sounds of fin whales (*Balaenoptera physalus*). However, the possibilities that the whales hear one another while migrating or while on a common summering area, that some singers visit more than one wintering ground during the same singing season, or that individual singers visit different wintering grounds in alternate winters are not so easily dismissed. Whatever its merits, the use of song similarities and differences to identify humpback stocks does not, as the authors conclude, "currently constitute the best and most practical technique for stock assessment of this species." In addition to the widely accepted technique of fluke matching, we believe that the analysis of catch histories and the different responses to overexploitation by regional populations can contribute to an understanding of stock identity. In the North Atlantic, for example, it appears on present evidence that humpbacks on the west side have made a strong recovery from overexploitation, while those on the east side have not. Humpbacks seem to be scarce at the Cape Verde Islands, where American whalers hunted them intensively during the nineteenth century. In contrast, they are abundant at the West Indies, where the same whalers hunted them during the same era. Surely it makes sense, in spite of the fact that the only song from the Cape Verdes analyzed thus far is similar to that sung by West Indies humpbacks, to manage humpbacks on either side of the North Atlantic as though they belonged to separate stocks (1984, Rep. Int. Whaling Comm. 34: 181).

The chapter in Part 4 on "External Features in Southern Right Whales (*Eubalaena australis*) and Their Use in Identifying Individuals" by R. Payne, Brazier, Dorsey, Perkins, Rowntree, and Titus is the most important in the book. It presents the rationale and methodology for identifying individual right whales with photographs. The long-term (12 yr and still running) program of research conducted by Payne and his associates at Peninsula Valdés, Argentina, depends upon the validity and routine application of this approach. The diagrams and textual definitions of various callosity sites on the right whale's head (e.g. bonnet, coaming, mandibular islands, etc.) will ensure that researchers studying right whales in other parts of the world have available (and hopefully adhere to) a common terminology.

Bird's bibliography has 390 entries for humpbacks and 194 entries for right whales. He supplies indexing rubrics but not annotations; so the bibliography is a classified rather than an annotated one, as indicated in the title. Although separated into two bibliographies with their respective author, subject, geographic, and journal indices, the humpback and right whale sections contain some of the same titles. Most published citations duplicate coverage in *Zoological Record*. Considering that this bibliography takes up about a quarter of the book's entire bulk, a much more economic presentation would have been appropriate. Although in his introduction Bird states that "published abstracts of papers presented at scientific meetings" are included, he has in fact included many unpublished abstracts of papers presented at scientific meetings. As an example, the humpback section contains some 18 titles identified as abstracts of papers presented at the Humpback Whales of the Western North Atlantic Workshop in Boston, November 17–21, 1980. These abstracts were circulated unbound to the participants but certainly have not been "published" in even the loosest sense of this much-abused word. Readers wishing to secure copies of these titles would hardly know where to begin, as neither the venue (in this instance the New England Aquarium) nor the sponsors (the National Marine Fisheries Service) are identified.

The book is tightly bound in cloth, and the diagrams and maps are clear. However, the typeface looks like justified typewriting, and the photographs and halftones suffer from the poor quality of the paper. This is particularly unfortunate in cases where the information in photographs serves as essential data. Figures 2 and 3 of Chapter 7, for example, appear to have lost much in the processes of reduction and printing.

The authors of parts of this book have presented most of the major findings in lectures at conferences, workshops, and

symposiums over the years. At least one chapter (12) has been available for some time as a contract report from the National Technical Information Service in Springfield, Virginia. The formal presentation of all the papers here under one cover is nevertheless highly appropriate and a welcome addition to the literature of cetology. With *Behavior of Marine Animals: Current Perspectives in Research, Volume 3: Cetaceans* edited by H. E. Winn and B. L. Olla (Plenum Publishing Corp., New York, NY, 1979) and *Cetacean Behavior: Mechanisms and Functions* edited by L. M. Herman (John Wiley & Sons, New York, NY, 1980), this book can be said to place whale behavior on a firm quantitative footing.

EDWARD MITCHELL AND RANDALL R. REEVES

Department of Fisheries and Oceans
Arctic Biological Station
555 St. Pierre Blvd.
Ste-Anne-de-Bellevue, Que. H9X 3R4 (R568)

BOOKS RECEIVED/LIVRES REÇUS

Diseases of marine animals. Volume IV, Part 1. Introduction, Pisces. *Otto Kinne (editor). Biologische Anstalt Helgoland, Hamburg. 1984. 541 p. DM 75 (including postage). ISBN 3-9800818-3-4.* In this volume the Editor summarizes and evaluates knowledge on the diseases of vertebrates that live either permanently or temporarily in marine or brackish habitats. The volume centers on biotic diseases, proliferative disorders, structural abnormalities, and impairments from environmental stressors. Part 1 covers the diseases of fish and is organized around the agents and hosts of the diseases. [R676]

A northern prairie stream. *Joe Kendall Nell, Sr. University of North Dakota Press, Grand Forks, ND. 1985. 274 p. $15.00.* Results of a decade of study of an intensively formed watershed in eastern North Dakota, from the effect of beaver dams on the river channel to changes in water chemistry. Biological studies deal mainly with attached plants and macrobenthos inhabitating riffles. [R678]

Fish diseases — Enfermedades de los Peces. Fourth COPRAQ Session. *ACUIGRUP (editor). ATP, S. A. Madrid-20, Spain. 1984. ISBN: 84-3982660-5.* This is the proceedings of the fourth session of COPRAQ (Cooperative Programme of Research on Aquaculture) held in October 1981 in Spain. Topics covered include viral, bacterial, and parasitic diseases as well as emerging studies of problems in marine cultures. The papers discuss (1) etiological and epizoological aspects of diseases and (2) practical methods of prophylaxis and treatment. [R680]

Grundlagen der Fischpathologie: mit einer Einfuhrung in Die Anatomie, Physiologie, Pathophysiologie und Immunologie sowie in den aquatischen Lebensraum der Knochenfische. *Edited by Ronald J. Roberts; translated and revised by Hans-Jürgen Schlotfeldt. Paul Parey, Berlin 61 (West) and Hamburg. 1985. ISBN 07020 06742. (In German).* This is a translation of the book *Fish Pathology* published in 1978 by Baillière Tindall (ISBN 07020 06742). [R681]

The Lammas Drave and the winter herrin': a history of the herring fishing from East Fife. *Peter Smith. John Donald Publishers Ltd., Edinburgh, Scotland. 1985. 166 p. £5.95. ISBN 0 85976 125 8.* This book traces the rise and fall of two local herring fishings in the Firth of Forth. The Lammas Drave peaked in 1860 and the winter herring fishing in 1936. In addition to the statistics on catches and fleets, there is information on the development of the fishing boats and the lines of the fishermen. [R682]

Ecosystem theory for biological oceanography. *Robert E. Ulanowicz and Trevor Platt (editors). Canadian Bulletin of Fisheries and Aquatic Sciences 213. 1985. 260 p. $14.95 (Canada); $17.95 (Other countries). ISBN 0-660-11900-5, ISSN 0706-6503.* This work presents a holistic perspective on marine ecosystems. Five subject areas are considered: thermodynamics, statistical mechanics, ataxonomic aggregations, flow analysis, and information theory. The book is based on a colloquium which took place in March 1984. [R683]

Status of the steller sea lion (*Eumetopias jubatus*) and California sea lion (*Zalophus californianus*) in British Columbia. *Michael A. Bigg. Canadian Special Publication of Fisheries and Aquatic Sciences 77. 1985. 20 p. $5.00 (Canada); $6.00 (Other countries). ISBN 0-660-11899-8, ISSN 0706-6481.* Using a report of aerial censures (1971–84) and a review of published and unpublished material since 1913 the author describes haulout sites, seasonal movements, trends in numbers, etc. [R684]

The determination of environmental levels of uranium and thorium series isotopes and ^{137}Cs in aquatic and terrestrial samples. *P. Wilkinson. Canadian Special Publication of Fisheries and Aquatic Sciences 78. 1985. 51 p. $4.00 (Canada); $4.80 (Other countries). ISBN 0-660-11829-7, ISSN 0706-6481.* This booklet details the analytical methods used for the radiochemical analysis of aquatic and terrestrial samples at the Freshwater Institute, Winnipeg, Manitoba. A separate section contains the mathematical formulae used to determine isotopes' absolute activity. [R685]

The enhancement of salmon stocks. Workshop at University of Surrey, Guildford, 9–11 April 1984. *Compiled by E. D. Le Cren. Atlantic Salmon Trust Ltd., Pitlochry, Perthshire. 1985. £1.50.* Discussions of progress in efforts to promote the preservation and enhancement of the stocks. [R686]

Salmon stocks: a genetic perspective. *N. P. Wilkins. Atlantic Salmon Trust Ltd., Pitlochry, Perthshire. 1985. 30 p. £1.50.* An account of current knowledge about the genetics of salmon. [R687]

ANNOUNCEMENTS/AVIS

Third Annual Meeting of the Aquaculture Association of Canada

4–7 June 1986
Guelph, Ontario

First Announcement and Call for Papers

The Third Annual Meeting of the Aquaculture Association of Canada (AAC) will be held at the University of Guelph, Guelph, Ont., from June 4 to 7, 1986. The program will include invited speakers and submitted papers (oral and poster).

Two special symposia or workshops are planned: Canada's Contribution to Aquaculture in Developing Countries, and Quality and Marketing of Aquaculture Products.

Topics for technical and business related papers are welcome in all areas relating to aquaculture and will include the following:

Engineering and Technological Development
Financial and Economic Considerations
Nutrition
Disease
Genetics
Education and Training
Seedstock Supply

Commercial aquaculturalists are particularly encouraged to submit papers on topics they feel would be of interest.

For further information, contact Gary Newkirk, 1986 AAC Program, Biology Department, Dalhousie University, Halifax, N.S. B3H 4J1.

The AAC will hold a Trade Show during the Annual Meeting. Those persons interested in displaying products, services, equipment, etc., to the aquaculture community should send their name and address to Nigel Robbins, Trillium Springs Fish Farm, R.R. 2, Holland Centre, Ont. N0H 1R0.

Troisième réunion annuelle de l'Association aquicole du Canada

4 au 7 juin 1986
Guelph (Ontario)

Première annonce et demande d'articles

La Troisième réunion annuelle de l'Assoiation aquicole du Canada (AAC) se tiendra à l'Université de Guelph, Guelph (Ont.), du 4 au 7 juin 1986. Le programme comprendra des conférenciers invités et des articles soumis (oraux et affiches).

Deux symposia ou ateliers spéciaux sont prévus : La contribution du Canada en aquiculture dans les pays en voie de développement et Qualité et marketing des produits aquicoles.

Les articles à thème technique ou commercial sont bienvenus dans tout les domaines ayant rapport a l'aquicole et seront :

Développement technologique et génie aquicole
Considérations économique et financières
Nutition
Maladie
Génétique
Éducation et formation
Approvisionnement en stocks reproducteurs

Les aquiculteurs sont encouragés à soumettre des articles sur les sujets qu'ils pensent être d'intérêt.

Pour plus d'information, communiquez avec Gary Newkirk, 1986 AAC Program, Département de biologie, Université de Dalhousie, Halifax (N.-É.) B3H 4J1.

L'AAC tiendra une exposition de commerce durant la réunion annuelle. Ceux qui sont intéressés à présenter leurs produits, services, équipements, etc., à la communauté aquicole devraient envoyer leur nom et adresse à Nigel Robbins, Trillium Springs Fish Farm, R.R. 2, Holland Centre (Ont.) N0H 1R0.

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Articles

Lake use by wild anadromous Atlantic salmon, *Salmo salar*, as an index of subsequent adult abundance
P. M. Ryan

Effects of temperature and food quality on growth and development of a mayfly, *Leptophlebia intermedia*
B. W. Sweeney, R. L. Vannote, and P. J. Dodds

Influence of dietary protein and lipid on apparent heat increment of rainbow trout, *Salmo gairdneri*
S. M. LeGrow and F. W. H. Beamish

Sexual differentiation and use of hormones to control sex in yellow perch (*Perca flavescens*)
J. A. Malison, T. B. Kayes, C. D. Best, C. H. Amundson, and B. C. Wentworth

Pathogenesis of experimental *Edwardsiella ictaluri* infections in channel catfish (*Ictalurus punctatus*)
E. B. Shotts, V. S. Blazer, and W. D. Waltman

Uptake of chrysotile asbestos fibers alters growth and reproduction of Asiatic clams
S. E. Belanger, D. S. Cherry, and J. Cairns, Jr.

High-density culture of meiobenthic harpacticoid copepods within a muddy sediment substrate
G. T. Chandler

Response of radioactive trace metals to acid–base titrations in controlled experimental ecosystems: evaluation of transport parameters for application to whole-lake radiotracer experiments
P. H. Santschi, U. P. Nyffeler, R. F. Anderson, S. L. Schiff, P. O'Hara, and R. H. Hesslein

Fundy tidal power development and potential fish production in the Gulf of Maine
D. E. Campbell and J. S. Wroblewski

Optimal effort allocation among competing mixed-species fisheries, subject to fishing mortality constraints
S. A. Murawski and J. T. Finn

Bounds on the optimal age-at-first-capture for stochastic, age-structured fisheries **W. S. Lovejoy**

Identifying the stock–recruitment relationship for age-structured populations using time-invariant matched linear filters **D. W. Welch**

Biogeographic influences on fish species composition of northern Wisconsin lakes with applications for lake acidification studies **F. J. Rahel**

Optimal stock size and harvest rate in multistage life history models **E. Moussalli and R. Hilborn**

Body fat condition in northeast Atlantic fin whales, *Balaenoptera physalus*, and its relationship with reproduction and food resource **C. Lockyer**

Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton **V. H. Smith**

Recent major declines in zooplankton populations in the inshore region of Lake Michigan: probable causes and implications **M. S. Evans**

Individual parameter perturbation and error analysis of fish bioenergetics models
S. M. Bartell, J. E. Breck, R. H. Gardner, and A. L. Brenkert

Effect of Aroclor 1254 and Mirex on estradiol-induced vitellogenin production in juvenile rainbow trout (*Salmo gairdneri*) **T. T. Chen, P. C. Reid, R. Van Beneden, and R. A. Sonstegard**

Analyzing catch–effort data allowing for randomness in the catching process **W. J. Reed**

Growth decline in subyearling brook trout (*Salvelinus fontinalis*) after emergence of rainbow trout (*Salmo gairdneri*) **G. A. Rose**

Size-limited predation, winterkill, and the organization of *Umbra–Perca* fish assemblages
W. M. Tonn and C. A. Paszkowski

Modification de la bioaccumulation du sélénium chez *Mytilus edulis* en présence du mercure organique et inorganique **E. Pelletier**

Ecological significance of the ctenophore *Pleurobrachia pileus* off southwestern Nova Scotia
K. T. Frank

Notes

Activity differences influencing food selection in the marine fish *Pomatoschistus microps* **C. Magnhaden**

Aluminum speciation in some synthetic systems: comparison of the fast-oxine, pH 5.0 extraction and dialysis methods **H. Lalande and W. H. Hendershot**

Importance of picoplankton in Lake Superior
G. L. Fahnenstiel, L. Sicko-Goad, D. Scavia, and E. F. Stoermer

Histological changes in cultured lake trout, *Salvelinus namaycush*, subjected to cumulative loading in a water reuse system **J. W. Meade and R. L. Herman**
Sports angler preferences for alternative regulatory methods **T. S. Renyard and R. Hilborn**
Acclimation-induced change in toxicity of aluminum to rainbow trout (*Salmo gairdneri*) **P. L. Orr, R. W. Bradley, J. B. Sprague, and N. J. Hutchinson**

Book reviews/Comptes rendus

Nitrogen in the marine environment (edited by Edward J. Carpenter and Douglas G. Capone) **K. Banse**

Books received/Livres reçus

Perspectives

Seaweeds, sea urchins, and lobsters: a reappraisal	R. J. Miller	**2061–2072**

Book reviews/Comptes rendus

QSAR in environmental toxicology (edited by L. E. Kaiser)	D. J. Ecobichon	**2073**
Proceedings of the National Aquaculture Conference. Strategies for aquaculture development in Canada (edited by G. I. Pritchard)	J. Hynes and A. Castledine	**2073–2074**
Storm surges — meteorological ocean tides (T. S. Murty)	P. F. Hamblin	**2074–2075**
Communication and behavior of whales (edited by Roger Payne)	E. Mitchell and R. R. Reeves	**2075–2077**

Books received/Livres reçus	**2078**
Announcements/Avis	**2079**
Coming next month/À paraître le mois prochain	**2080–2081**

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 42, No. 12, December 1985

Volume 42, nº 12, décembre 1985

Articles

Estimating the standing biomass of aquatic macrophytes J. A. Downing and M. A. Anderson **1860–1869**

Interactive effects of cadmium, polychlorinated biphenyls, and fuel oil on experimentally exposed English sole (*Parophrys vetulus*) L. Rhodes, E. Casillas, B. McKnight, W. Gronlund, M. Myers, P. Olson, and B. McCain **1870–1880**

Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in *Chironomus* Meigen W. F. Warwick **1881–1914**

Foraging behavior of hatchery-produced coho salmon (*Oncorhynchus kisutch*) smolts on live prey C. A. Paszkowski and B. L. Olla **1915–1921**

Effects of successive flow perturbations on stream invertebrates J. R. Irvine **1922–1927**

Influence of mesh composition, velocity, and run time on the catch and length composition of juvenile alewives (*Alosa pseudoharengus*) and blueback herring (*A. aestivalis*) collected by pushnet B. M. Jessop **1928–1939**

Biological control of excessive phytoplankton growth and the enhancement of aquacultural production D. W. Smith **1940–1945**

Effects of manipulations of aluminum concentrations and pH on phosphate uptake and photosynthesis of planktonic communities in two Precambrian Shield lakes C. Nalewajko and B. Paul **1946–1953**

Fertilization and egg retention in artificially inseminated female American lobsters, *Homarus americanus* S. L. Waddy and D. E. Aiken **1954–1956**

Potential of parasites for use as biological indicators of migration, feeding, and spawning behavior of northwestern Atlantic herring (*Clupea harengus*) S. E. McGladdery and M. D. B. Burt **1957–1968**

Variabilité interannuelle d'un indice d'intensité des remontées d'eau dans le secteur du cap Blanc (Mauritanie) R. Arfi **1969–1978**

Influence of snowcover development and ground freezing on cation loss from a wetland watershed during spring runoff D. C. Pierson and C. H. Taylor **1979–1985**

Potential usefulness of chlorine for controlling Pacific salmon leeches, *Piscicola salmositica*, in hatcheries S. M. Bower, L. Margolis, and R. J. McKay **1986–1993**

Comparative analysis of ionoregulation in rainbow trout (*Salmo gairdneri*) of different sizes following rapid and slow salinity adaptation C. E. Johnston and J. C. Cheverie **1994–2003**

Physiological response of juvenile rainbow trout, *Salmo gairdneri*, to acid and aluminum — prediction of field responses from laboratory data C. M. Neville **2004–2019**

Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch* E. B. Taylor and J. D. McPhail **2020–2028**

Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch* E. B. Taylor and J. D. McPhail **2029–2033**

Reviews/Synthèses

Acidification and toxicity of metals to aquatic biota P. G. C. Campbell and P. M. Stokes **2034–2049**

Notes

The right to fish as an external benefit of open access R. E. Townsend **2050–2053**

Removal of follicle wall components from ovarian oocytes of the brook trout, *Salvelinus fontinalis* C. A. Lessman, S. Kavumpurath, and H. R. Habibi **2053–2058**

Changes to Stock Reduction Analysis indicated by Schnute's general theory D. K. Kimura **2059–2060**

(*continued inside back*)

CJFSDX42(12) 1859–2081 (1985)
ISSN 0706-652X
© Minister of Supply and Services Canada 1985
Ministre des Approvisionnements et Services Canada 1985

Subscription rates/Abonnements
Canada $69.30
Other countries/Autres pays $83.15